PB92-207703

# Methods for the Determination of Organic Compounds in Drinking Water. Supplement 2

(U.S.) Environmental Monitoring Systems Lab., Cincinnati, OH

Aug 92

U.S. Department of Commerce
National Technical Information Service
NTIS

## TECHNICAL REPORT DATA

*(Please read Instructions on the reverse before compl*

**1. REPORT NO.** EPA/600/R-92/129

**2.**

PB92-207703

**4. TI**
"Methods for the Determination of Organic
Compounds in Drinking Water, Supplement II"

**5. REPORT DATE** August 1992

**6. PERFORMING ORGANIZATION CODE**

**7. AUTHOR(S)**
Chemistry Research Division
Environmental Monitoring Systems Laboratory - Cincinnati

**8. PERFORMING ORGANIZATION REPORT NO.**

**9. PERFORMING ORGANIZATION NAME AND ADDRESS**
Chemistry Research Division
Environmental Monitoring Systems Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

**10. PROGRAM ELEMENT NO.**

**11. CONTRACT/GRANT NO.** -

**12. SPONSORING AGENCY NAME AND ADDRESS**
Same as 9.

**13. TYPE OF REPORT AND PERIOD COVERED** EPA Report

**14. SPONSORING AGENCY CODE** 600 R92-129

**15. SUPPLEMENTARY NOTES**

**16. ABSTRACT**

Eight analytical methods covering 133 organic contaminants which may be present in drinking water or drinking water sources are described in detail. These methods will give the laboratory analyst the capability to accurately and precisely determine organic compounds currently regulated in drinking water, designated for regulation by the Office of Ground Water and Drinking Water in the near future, or are potential candidates for regulatory concern. The only method to determine volatile organic compounds in this manual, Method 524.2, Revision 4.0, has 24 new analytes added to the method analyte list. Four of the eight methods are improvements over older existing methods, and employ new analytical techniques, such as liquid-solid extraction, to improve method performance and reduce the use of organic solvents. Three of the methods are new methods for the determination of semivolatile compounds, one for ozonation disinfection by-products, one for phenoxyacid herbicides using the novel approach of in-line extraction/high performance liquid chromatography (HPLC), and one for nonvolatile compounds using particle beam HPLC/MS.

**17. KEY WORDS AND DOCUMENT ANALYSIS**

| a. DESCRIPTORS | b. IDENTIFIERS/OPEN ENDED TERMS | c. COSATI Field/Group |
|---|---|---|
| | | |

**18. DISTRIBUTION STATEMENT**
Release to Public

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL INFORMATION SERVICE
SPRINGFIELD, VA 22161

**19. SECURITY CLASS** *(This Report)* Unclassified

**20. SECURITY CLASS** *(This page)* Unclassified

**21. NO. OF PAGES** 270

**22. PRICE**

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

PB92-207703

EPA-600/R-92/129
AUGUST 1992

# METHODS FOR THE DETERMINATION OF ORGANIC COMPOUNDS IN DRINKING WATER

## SUPPLEMENT II

Environmental Monitoring Systems Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

Printed on Recycled Paper

# DISCLAIMER

This manual has been reviewed by the Environmental Monitoring Systems Laboratory - Cincinnati, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

# FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring Systems Laboratory - Cincinnati (EMSL-Cincinnati) conducts research to:

- o Develop and evaluate analytical methods to identify and measure the concentration of chemical pollutants in drinking waters, surface waters, groundwaters, wastewaters, sediments, sludges, and solid wastes.
- o Investigate methods for the identification and measurement of viruses, bacteria and other microbiological organisms in aqueous samples and to determine the responses of aquatic organisms to water quality.
- o Develop and operate a quality assurance program to support the achievement of data quality objectives in measurements of pollutants in drinking water, surface water, groundwater, wastewater, sediment and solid waste.
- o Develop methods and models to detect and quantify responses in aquatic and terrestrial organisms exposed to environmental stressors and to correlate the exposure with effects on chemical and biological indicators.

This publication, "Determination of Organic Compounds in Drinking Water Supplement II," was prepared to gather together under a single cover a set of eight new or improved laboratory analytical methods for organic compounds in drinking water. EMSL-Cincinnati is pleased to provide this manual and believe that it will be of considerable value to many public and private laboratories that wish to determine organic compounds in drinking water for regulatory or other reasons.

Thomas A. Clark, Director
Environmental Monitoring Systems
Laboratory - Cincinnati

# ABSTRACT

Eight analytical methods covering 133 organic contaminants which may be present in drinking water or drinking water sources are described in detail. These methods will give the laboratory analyst the capability to accurately and precisely determine organic compounds currently regulated in drinking water, designated for regulation by the Office of Ground Water and Drinking Water in the near future, or are potential candidates for regulatory concern.

Five of the methods in this manual, Methods 515.2, 524.2 Revision 4.0, 548.1, 549.1, and 552.1, replace older versions of these methods. The older versions were numbered 515.1, 524.2 Revision 3.0, 548, 549, and 552, respectively, and appeared in either of two previous organic methods manuals[1]. The new versions employ new analytical techniques, such as liquid-solid extraction, to improve method performance and reduce the use of organic solvents. Three are new methods for the determination of semivolatile compounds: Method 554 for ozonation disinfection by-products, Method 555 for phenoxyacid herbicides using the novel approach of in-line extraction/high performance liquid chromatography (HPLC), and Method 553 for nonvolatile compounds using particle beam HPLC/MS.

[1] "Methods for the Determination of Organic Compounds in Drinking Water," EPA/600/4-88/039, December 1988, revised July 1991, or "Methods for the Determination of Organic Compounds in Drinking Water - Supplement I," EPA/600/4-90/020, July 1990; Environmental Monitoring Systems Laboratory - Cincinnati.

## TABLE OF CONTENTS

| Method Number | Title | Revision | Page |
|---|---|---|---|
| - | Foreword |  | iii |
| - | Abstract |  | iv |
| - | Acknowledgment |  | vi |
| - | Analyte - Method Cross Reference |  | vii |
| - | Introduction |  | 1 |
| 524.2 | Measurement of Purgeable Organic Compounds in Water by Capillary Column Gas Chromatography/ Mass Spectrometry | 4.0 | 5 |
| 515.2 | Determination of Chlorinated Acids in Water Using Liquid-Solid Extraction and Gas Chromatography With an Electron Capture Detector | 1.0 | 51 |
| 548.1 | Determination of Endothall in Drinking Water by Ion Exchange Extraction, Acidic Methanol, Methylation Gas Chromatography/Mass Spectrometry | 1.0 | 89 |
| 549.1 | Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and HPLC with Ultraviolet Detection | 1.0 | 119 |
| 552.1 | Determination of Haloacetic Acids and Dalapon in Drinking Water by Ion Exchange Liquid-Solid Extraction and Gas Chromatography With Electron Capture Detection | 1.0 | 143 |
| 553 | Determination of Benzidines and Nitrogen-Containing Pesticides in Water by Liquid-Liquid Extraction or Liquid-Solid Extraction and Reverse Phase High Performance Liquid Chromatography/Particle Beam/Mass Spectrometry | 1.1 | 173 |
| 554 | Determination of Carbonyl Compounds in Drinking Water by DNPH Derivatization and High Performance Liquid Chromatography | 1.0 | 213 |
| 555 | Determination of Chlorinated Acids in Water by High Performance Liquid Chromatography With a Photodiode Array Ultraviolet Detector | 1.0 | 237 |

# ACKNOWLEDGMENT

This methods manual was prepared and assembled by the Organic Chemistry Branch of the Chemistry Research Division, Environmental Monitoring Systems Laboratory - Cincinnati. Special thanks and appreciation are due to Glenora F. Green for providing outstanding secretarial and word processing support, for format improvements in presentation of the material in the manual, and for coordinating the final assembly of the manual.

In addition, William L. Budde, Director of the Chemistry Research Division, is recognized for his significant contributions. James W. Eichelberger supervised the development of the methods, reviewed and edited each of the individual methods, and directed the publication of the manual. Dr. Jimmie Hodgeson managed the research which led to the successful development of three of the methods and prepared the method descriptions. Thomas A. Bellar, Thomas D. Behymer, and James S. Ho conducted the research in particle beam high performance liquid chromatography (HPLC) mass spectrometry which led to the development of Method 553. Jean W. Munch conducted the research which led to the addition of 24 new volatile organic compounds to the analyte list in Method 524.2 Revision 4.0. Jeffery D. Collins, David Becker, and Winslow J. Bashe, Technology Applications, Inc., performed the major portion of the laboratory support necessary to develop five of the methods. Appreciation is also extended to the scientists in the Technical Support Division of the Office of Ground Water and Drinking Water for their constructive and beneficial review of the analytical methods contained in this manual.

The Quality Assurance Research Division of the Environmental Monitoring Systems Laboratory - Cincinnati also assisted by providing sound constructive reviews of many of the methods.

Finally, all the method authors and contributors wish to thank the administrators and managers of the Environmental Protection Agency, who supported the development and preparation of this manual. Special appreciation is due to Thomas A. Clark, Director of the Environmental Monitoring Systems Laboratory - Cincinnati, for his cooperation and support during this project.

## ANALYTE - METHOD CROSS REFERENCE

| Analyte | Method No. |
|---|---|
| Acetaldehyde | 554 |
| Acetone | 524.2 |
| Acifluorofen | 515.2, 555 |
| Acrylonitrile | 524.2 |
| Allyl chloride | 524.2 |
| Bentazon | 515.2, 555 |
| Benzene | 524.2 |
| Benzidine | 553 |
| Benzoylprop ethyl | 553 |
| Bromobenzene | 524.2 |
| Bromochloroacetic acid | 552.1 |
| Bromochloromethane | 524.2 |
| Bromodichlorobenzene | 524.2 |
| Bromoform | 524.2 |
| Bromomethane | 524.2 |
| Butanal | 554 |
| 2-Butanone | 524.2 |
| n-Butylbenzene | 524.2 |
| sec-Butylbenzene | 524.2 |
| tert-Butylbenzene | 524.2 |
| Carbaryl | 553 |
| Carbon disulfide | 524.2 |
| Carbon tetrachloride | 524.2 |
| Chloramben | 515.2, 555 |
| Chloroacetonitrile | 524.2 |
| Chlorobenzene | 524.2 |
| 1-Chlorobutane | 524.2 |
| Chloroethane | 524.2 |
| Chloroform | 524.2 |
| Chloromethane | 524.2 |
| 2-Chlorotoluene | 524.2 |
| 4-Chlorotoluene | 524.2 |
| Crotonaldehyde | 554 |
| Cyclohexanone | 554 |
| Dalapon | 552.1 |
| DCPA (Dacthal) and metabolites | 515.2, 555 |
| Decanal | 554 |
| Dibromoacetic acid | 552.1 |
| Dibromochloromethane | 524.2 |
| 1,2-Dibromo-3-chloropropane | 524.2 |
| 1,2-Dibromoethane | 524.2 |
| Dibromomethane | 524.2 |
| Dicamba | 515.2, 555 |
| Dichloroacetic acid | 552.1 |
| 1,2-Dichlorobenzene | 524.2 |
| 1,3-Dichlorobenzene | 524.2 |

| **Analyte** | **Method No.** |
|---|---|
| 1,4-Dichlorobenzene | 524.2 |
| 3,3'-Dichlorobenzidine | 553 |
| 3,5-Dichlorobenzoic acid | 515.2, 555 |
| 2,4-DB (2,4-dichlorobutanoic acid) | 515.2, 555 |
| trans-1,4-Dichloro-2-butene | 524.2 |
| Dichlorodifluoromethane | 524.2 |
| 1,1-Dichloroethane | 524.2 |
| 1,2-Dichloroethane | 524.2 |
| 1,1-Dichloroethene | 524.2 |
| cis-1,2-Dichloroethene | 524.2 |
| trans-1,2-Dichloroethene | 524.2 |
| 2,4-D (2,4-dichlorophenoxyacetic acid) | 515.2, 555 |
| 1,2-Dichloropropane | 524.2 |
| 1,3-Dichloropropane | 524.2 |
| 2,2-Dichloropropane | 524.2 |
| 1,1-Dichloropropene | 524.2 |
| 1,1-Dichloropropanone | 524.2 |
| cis-1,3-Dichloropropene | 524.2 |
| trans-1,3-Dichloropropene | 524.2 |
| Dichlorprop | 515.2, 555 |
| Diethyl ether | 524.2 |
| 3,3'-Dimethoxybenzidine | 553 |
| 3,3'-Dimethylbenzidine | 553 |
| Dinoseb | 515.2, 555 |
| Diquat | 549.1 |
| Diuron | 553 |
| Endothall | 548.1 |
| Ethylbenzene | 524.2 |
| Ethyl methacrylate | 524.2 |
| Formaldehyde | 554 |
| Heptanal | 554 |
| Hexachlorobutadiene | 524.2 |
| Hexachloroethane | 524.2 |
| Hexanal | 554 |
| 2-Hexanone | 524.2 |
| 5-Hydroxydicamba | 515.2, 555 |
| Isopropylbenzene | 524.2 |
| 4-Isopropyltoluene | 524.2 |
| Linuron (Lorox) | 553 |
| Methacrylonitrile | 524.2 |
| Methylacrylate | 524.2 |
| Methylene chloride | 524.2 |
| Methyl iodide | 524.2 |
| Methylmethacrylate | 524.2 |
| 4-Methyl-2-pentanone | 524.2 |
| Methyl-t-butyl ether | 524.2 |
| Monobromoacetic acid | 552.1 |
| Monochloroacetic acid | 552.1 |

| **Analyte** | **Method No.** |
|---|---|
| Monuron | 553 |
| Naphthalene | 524.2 |
| Nitrobenzene | 524.2 |
| 4-Nitrophenol | 515.2, 555 |
| 2-Nitropropane | 524.2 |
| Nonanal | 554 |
| Octanal | 554 |
| Paraquat | 549.1 |
| Pentachloroethane | 524.2 |
| Pentachlorophenol (PCP) | 515.2, 555 |
| Pentanal | 554 |
| Picloram | 515.2, 555 |
| Propanal | 554 |
| Propionitrile | 524.2 |
| n-Propylbenzene | 524.2 |
| Rotenone | 553 |
| Siduron | 553 |
| 2,4,5-TP (silvex) | 515.2, 555 |
| Styrene | 524.2 |
| Tetrachloroethene | 524.2 |
| 1,1,1,2-Tetrachloroethane | 524.2 |
| 1,1,2,2-Tetrachloroethane | 524.2 |
| Tetrahydrofuran | 524.2 |
| Toluene | 524.2 |
| Trichloroacetic acid | 552.1 |
| 1,2,3-Trichlorobenzene | 524.2 |
| 1,2,4-Trichlorobenzene | 524.2 |
| Trichloroethene | 524.2 |
| 1,1,1-Trichloroethane | 524.2 |
| 1,1,2-Trichloroethane | 524.2 |
| Trichlorofluoromethane | 524.2 |
| 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) | 515.2, 555 |
| 1,2,3-Trichloropropane | 524.2 |
| 1,2,4-Trimethylbenzene | 524.2 |
| 1,3,5-Trimethylbenzene | 524.2 |
| Vinyl chloride | 524.2 |
| m-Xylene | 524.2 |
| o-Xylene | 524.2 |
| p-Xylene | 524.2 |

# INTRODUCTION

An integral component of the role of the Environmental Protection Agency (EPA) in protecting the quality of the Nation's water resources is the provision of means for monitoring water quality. In keeping with this role, EPA develops and disseminates analytical methods for measuring chemical and physical parameters affecting water quality, including chemical contaminants that may have potential adverse effects upon human health. This manual provides eight analytical methods for 133 organic contaminants, which may be present in drinking water or drinking water sources. In December 1988, EPA published "METHODS FOR THE DETERMINATION OF ORGANIC COMPOUNDS IN DRINKING WATER," EPA/600/4-88/039, a manual containing 13 methods for approximately 200 potential drinking water contaminants. This original manual was revised and reprinted in July 1991. Supplement I, containing nine methods to determine 54 compounds, was published in July 1990.

This manual is a second supplement to the July 1991 revision of the earlier 1988 manual. This manual provides methods to determine analytes that appear at a later time in the regulatory framework, and technology that supports the EPA Pollution Prevention Policy. Efforts have been made to provide a manual that is consistent with the earlier versions.

## REGULATORY BACKGROUND

Analytical methodology for monitoring water quality serves a number of related purposes, including occurrence studies in community water systems, health effects studies, and the determination of the efficacy of various water treatment approaches. These activities, in turn, form the supporting basis for water quality regulations, and the support of these regulations is the ultimate purpose of the analytical methods. Limitations on the levels of specific contaminants are codified in proposed and promulgated Federal regulations developed in response to the Safe Drinking Water Act (SDWA) of 1974 and the SDWA amendments of 1986. The Act requires EPA to promulgate regulations for drinking water contaminants that may cause adverse health effects and which are known or anticipated to occur in public water systems. The 1986 amendments require regulations to include Maximum Contaminant Levels (MCLs) with compliance determined by regulatory monitoring or by the application of an appropriate treatment, when adequate analytical methodology is not available. In addition, the 1986 amendments specified 83 contaminants, originally scheduled for regulation by June 19, 1989. The amended Act also required EPA to develop a priority list of additional contaminants, to propose 25 more of these by January 1988 for subsequent regulation, and to continue this process by the addition of 25 from the priority list on a triennial basis thereafter.

Of the original 83 pollutants, regulations for eight volatile organic chemicals (VOCs) were promulgated in June 1987 (see 52 FR 25690 and 51 FR 11396). Analytical methods for these eight as well as other unregulated VOCs were published in the December 1988 manual (EPA Methods 502.1, 502.2, 503.1, 524.1 and 524.2). Regulations for 30 organic chemicals, 10 volatile compounds and 20 pesticide and related compounds, were finalized and published in

January 1991. This group included six compounds which, by authority of provisions in the 1986 amendments, were substituted into the original list of 83 in January 1988 (53 FR 1892): namely, aldicarb sulfoxide, aldicarb sulfone, ethylbenzene, heptachlor, heptachlor epoxide and styrene. With the exception of lindane, analytical methods for all 30 compounds are by the VOC methods above or SOC methods also included in the 1988 manual.

Supplement I provides analytical methods for many of the remaining contaminants on the original list of 83: namely, adipates, diquat, endothall, glyphosate, polycyclic aromatic hydrocarbons (PAHs), phthalates and dioxin. Phase V of EPA regulations for 3 volatile compounds, 9 pesticides, and 6 other organics from the list of 83 was promulgated on July 17, 1992.

## GENERAL COMMENTS

Supplement II provides methods, which are cast in the same terminology as the December 1988 manual, the July 1991 revision, and Supplement I. The introductions to the earlier manuals discuss general method features on format, sample matrices, method detection limits (MDLs), and calibration and quality control samples. These same comments apply herein. In particular, these methods are written in standardized terminology in a stand-alone format, requiring no other source material for application. The methods in this manual, unlike previous manuals, are assembled in the format recommended by the Agency's Environmental Monitoring Management Council (EMMC). The methods are designed primarily for drinking water and drinking water sources. However, some performance data are included for more complex matrices such as wastewater. The MDLs provided were determined by replicate analyses of fortified reagent water over a relatively short period of time. As such, these are somewhat idealized limits; nevertheless, provide a useful index of method performance. Reporting limits for reliable quantitative data may be considerably higher.

The quality control sections are uniform and contain minimum requirements for operating a reliable monitoring program -- initial demonstration of performance, routine analyses of reagent blanks, analyses of fortified reagent blanks and fortified matrix samples, and analyses of quality control (QC) samples. Other QC practices are recommended and may be adopted to meet the particular needs of monitoring programs; e.g., the analyses of field reagent blanks, instrument control samples and performance evaluation samples. Where feasible, surrogate analytes have been included in the methods as well as internal standards for calibration. Surrogate recoveries and the internal standard response should be routinely monitored as continuing checks on instrument performance, calibration curves and overall method performance.

## THE ANALYTICAL METHODS

This manual contains eight methods. These methods utilize new sample preparation technology such as disk or cartridge liquid-solid extraction, or use new relatively harmless methylating reagents; therefore, directly support the Environmental Protection Agency's Policy on Pollution Prevention. Methods 515.2, 524.2 Revision 4.0, 548.1, 549.1, and 552.1 replace older versions of these methods. The older versions were numbered 515.1, 524.2 Revision 3.0, 548, 549, and 552 respectively, and appeared in either of the two previously published organic methods manuals ("Methods for the Determination of Organic Compounds in Drinking Water," EPA/600/4-88/039, December 1988, revised July 1991, or "Methods for the Determination of Organic Compounds in Drinking Water - Supplement I," EPA/600/4-90/020, July 1990; Environmental Monitoring Systems Laboratory - Cincinnati). The Environmental Protection Agency's Office of Ground Water and Drinking Water believes that only one version of any analytical method should be approved for compliance with drinking water regulations. Thus, EPA will quickly approve the new versions of these five methods. Until these methods are promulgated by EPA, the older versions should be retained for compliance purposes. The remaining three are new methods for the determination of semivolatile or nonvolatile compounds: Method 553 for nonvolatile organics using a high performance liquid chromatograph interfaced to a mass spectrometer through a particle beam interface, Method 554 for ozonation disinfection by-products, and Method 555 for phenoxyacetic acid herbicides using the novel approach of in-line liquid-solid extraction and high performance liquid chromatography.

Method 524.2, Revision 4.0 contains 24 new target analytes, which are marked in the analyte list with an asterisk, bringing the total number of method analytes to 84 compounds. Initial studies were conducted to evaluate 48 candidate VOCs of environmental interest for possible inclusion into this method. These candidate compounds included many polar, water soluble compounds which are very difficult to remove from the water matrix. Results indicated that only 24 of these candidates were stable in water over a 14-day holding time and could be efficiently purged and trapped from water with acceptable accuracy and precision. MDLs for these newly added compounds are generally 1 µg/L or lower.

Method 515.2 is an improved method to determine chlorinated herbicides in water. This method utilizes a new liquid-solid disk extraction procedure. Some of the phenolic herbicides are very difficult to derivatize, and still require the use of the stronger reagent, diazomethane. The disk extraction replaces the cumbersome liquid-liquid extraction and the Florisil cleanup in Method 515.2. Dalapon, a method analyte in Method 515.1, is no longer a method analyte in this new method. This compound is now determined using Method 552.1.

Method 548.1 is an improved method for the determination of endothall and is intended to replace the older Method 548. This method utilizes an intermediate strength amine anion exchange sorbent to extract the endothall from a 100 mL sample aliquot, and forms the dimethyl derivative quickly and easily using acidic methanol as the methylating reagent. Dimethyl endothall is then identified and measured with gas chromatography/mass spectrometry (GC/MS). A flame ionization detector may be used if a second dissimilar column is used for corroboration. Method 548.1 has a MDL approximately ten times lower than the older 548.

Method 549.1 is an improvement over the existing Method 549 in that it now utilizes liquid-solid disk extractions in addition to the cartridges. The expanded liquid-solid extraction technology significantly reduces the amount of organic solvent required to carry out the extraction. Using less potentially harmful solvents directly supports the Environmental Protection Agency's Policy on Pollution Prevention in the laboratory. Comparable MDLs below a part per billion are achieved with both disks and cartridges.

Method 552.1 is a liquid-solid extraction method to determine haloacetic acid disinfection by-products and the chemically similar chlorinated herbicide, dalapon, in water. This method was designed as a simplified alternative to the cumbersome Method 552 which employs liquid-liquid extraction. This method provides a much superior technique for Dalapon over the complex herbicide procedure described in Method 515.1. The sample is extracted with a miniature anion exchange column, and the analytes are methylated directly in the eluant using acidic methanol instead of diazomethane. MDLs using this method for matrices which pose no analyte losses due to matrix effects are generally 1 µg/L or lower.

Method 553 is a new method for the determination of nonvolatile organic compounds, including benzidines and nitrogen containing pesticides, in water. This method employs reverse phase high performance liquid chromatography (HPLC) interfaced to a mass spectrometer through a particle beam interface. This new technology provides the analyst the ability to determine a large, new scope of nonvolatiles heretofore extremely difficult or impossible to determine in a water matrix. Among the compounds on the analyte list for this method are two regulated compounds, aldicarb sulfone regulated in January 1991 and carbofuran regulated in May 1992.

Method 554 is a new HPLC method optimized for the determination of selected carbonyl compounds in finished drinking water and raw source water. These carbonyl compounds are either known or suspected disinfection by-products from the ozonation disinfecting process. This method also utilizes cartridge liquid-solid extraction technology in support of the Agency's Pollution Prevention Policy. MDLs for the analytes in this method range from 3 to 69 µg/L.

Method 555 is a new method utilizing HPLC with a conventional photodiode array HPLC detector to determine the same chlorinated herbicides on the analyte lists in Methods 515.1 and 515.2. This method requires no derivatization procedure which completely eliminates the need for diazomethane or even acidic methanol. This method utilizes a new extraction approach, in-line concentration of the analytes on a concentrator column, which requires only the HPLC mobile phase as the extracting solvent. The entire amount of herbicide contained in a 20-mL sample aliquot is introduced into the analytical system giving the method the needed sensitivity. This method directly supports the Pollution Prevention Policy, and completely eliminates the possible exposure of the analyst to harmful extracting solvent vapors, and to the possibility of a diazomethane explosion.

# METHOD 524.2. MEASUREMENT OF PURGEABLE ORGANIC COMPOUNDS IN WATER BY CAPILLARY COLUMN GAS CHROMATOGRAPHY/MASS SPECTROMETRY

Revision 4.0

August 1992

A. Alford-Stevens, J. W. Eichelberger, and W. L. Budde
Method 524, Revision 1.0 (1983)

R. W. Slater, Jr.
Revision 2.0 (1986)

J. W. Eichelberger, and W. L. Budde
Revision 3.0 (1989)

J. W. Eichelberger, J. W. Munch, and T. A.Bellar
Revision 4.0 (1992)

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

# METHOD 524.2

# MEASUREMENT OF PURGEABLE ORGANIC COMPOUNDS IN WATER BY CAPILLARY COLUMN GAS CHROMATOGRAPHY/MASS SPECTROMETRY

## 1. SCOPE AND APPLICATION

1.1 This is a general purpose method for the identification and simultaneous measurement of purgeable volatile organic compounds in surface water, ground water, and drinking water in any stage of treatment (1,2). The method is applicable to a wide range of organic compounds, including the four trihalomethane disinfection by-products, that have sufficiently high volatility and low water solubility to be removed from water samples with purge and trap procedures. The following compounds can be determined by this method.

| Compound | Chemical Abstract Service Registry Number |
|---|---|
| Acetone* | 67-64-1 |
| Acrylonitrile* | 107-13-1 |
| Allyl chloride* | 107-05-1 |
| Benzene | 71-43-2 |
| Bromobenzene | 108-86-1 |
| Bromochloromethane | 74-97-5 |
| Bromodichloromethane | 75-27-4 |
| Bromoform | 75-25-2 |
| Bromomethane | 74-83-9 |
| 2-Butanone* | 78-93-3 |
| n-Butylbenzene | 104-51-8 |
| sec-Butylbenzene | 135-98-8 |
| tert-Butylbenzene | 98-06-6 |
| Carbon disulfide* | 75-15-0 |
| Carbon tetrachloride | 56-23-5 |
| Chloroacetonitrile* | 107-14-2 |
| Chlorobenzene | 108-90-7 |
| 1-Chlorobutane* | 109-69-3 |
| Chloroethane | 75-00-3 |
| Chloroform | 67-66-3 |
| Chloromethane | 74-87-3 |
| 2-Chlorotoluene | 95-49-8 |
| 4-Chlorotoluene | 106-43-4 |
| Dibromochloromethane | 124-48-1 |
| 1,2-Dibromo-3-chloropropane | 96-12-8 |
| 1,2-Dibromoethane | 106-93-4 |
| Dibromomethane | 74-95-3 |
| 1,2-Dichlorobenzene | 95-50-1 |
| 1,3-Dichlorobenzene | 541-73-1 |
| 1,4-Dichlorobenzene | 106-46-7 |
| trans-1,4-Dichloro-2-butene* | 110-57-6 |
| Dichlorodifluoromethane | 75-71-8 |

| | |
|---|---|
| 1,1-Dichloroethane | 75-34-3 |
| 1,2-Dichloroethane | 107-06-2 |
| 1,1-Dichloroethene | 75-35-4 |
| cis-1,2-Dichloroethene | 156-59-4 |
| trans-1,2-Dichloroethene | 156-60-5 |
| 1,2-Dichloropropane | 78-87-5 |
| 1,3-Dichloropropane | 142-28-9 |
| 2,2-Dichloropropane | 590-20-7 |
| 1,1-Dichloropropene | 563-58-6 |
| 1,1-Dichloropropanone* | 513-88-2 |
| cis-1,3-Dichloropropene | 10061-01-5 |
| trans-1,3-Dichloropropene | 10061-02-6 |
| Diethyl ether* | 60-29-7 |
| Ethylbenzene | 100-41-4 |
| Ethyl methacrylate* | 97-63-2 |
| Hexachlorobutadiene | 87-68-3 |
| Hexachloroethane* | 67-72-1 |
| 2-Hexanone* | 591-78-6 |
| Isopropylbenzene | 98-82-8 |
| 4-Isopropyltoluene | 99-87-6 |
| Methacrylonitrile* | 126-98-7 |
| Methylacrylate* | 96-33-3 |
| Methylene chloride | 75-09-2 |
| Methyl iodide* | 74-88-4 |
| Methylmethacrylate* | 80-62-6 |
| 4-Methyl-2-pentanone* | 108-10-1 |
| Methyl-t-butyl ether* | 1634-04-4 |
| Naphthalene | 91-20-3 |
| Nitrobenzene* | 98-95-3 |
| 2-Nitropropane* | 79-46-9 |
| Pentachloroethane* | 76-01-7 |
| Propionitrile* | 107-12-0 |
| n-Propylbenzene | 103-65-1 |
| Styrene | 100-42-5 |
| 1,1,1,2-Tetrachloroethane | 630-20-6 |
| 1,1,2,2-Tetrachloroethane | 79-34-5 |
| Tetrachloroethene | 127-18-4 |
| Tetrahydrofuran* | 109-99-9 |
| Toluene | 108-88-3 |
| 1,2,3-Trichlorobenzene | 87-61-6 |
| 1,2,4-Trichlorobenzene | 120-82-1 |
| 1,1,1-Trichloroethane | 71-55-6 |
| 1,1,2-Trichloroethane | 79-00-5 |
| Trichloroethene | 79-01-6 |
| Trichlorofluoromethane | 75-69-4 |
| 1,2,3-Trichloropropane | 96-18-4 |
| 1,2,4-Trimethylbenzene | 95-63-6 |
| 1,3,5-Trimethylbenzene | 108-67-8 |
| Vinyl chloride | 75-01-4 |
| o-Xylene | 95-47-6 |
| m-Xylene | 108-38-3 |
| p-Xylene | 106-42-3 |

* New Compound in Revision 4.0

1.2 Method detection limits (MDLs) (3) are compound, instrument and especially matrix dependent and vary from approximately 0.02 to 1.6 µg/L. The applicable concentration range of this method is primarily column and matrix dependent, and is approximately 0.02 to 200 µg/L when a wide-bore thick-film capillary column is used. Narrow-bore thin-film columns may have a capacity which limits the range to about 0.02 to 20 µg/L. Volatile water soluble, polar compounds which have relatively low purging efficiencies can be determined using this method. Such compounds may be more susceptible to matrix effects, and the quality of the data may be adversely influenced.

1.3 Analytes that are not separated chromatographically, but which have different mass spectra and noninterfering quantitation ions (Table 1), can be identified and measured in the same calibration mixture or water sample as long as their concentrations are somewhat similar (Sect. 11.6.2). Analytes that have very similar mass spectra cannot be individually identified and measured in the same calibration mixture or water sample unless they have different retention times (Sect. 11.6.3). Coeluting compounds with very similar mass spectra, typically many structural isomers, must be reported as an isomeric group or pair. Two of the three isomeric xylenes and two of the three dichlorobenzenes are examples of structural isomers that may not be resolved on the capillary column, and if not, must be reported as isomeric pairs. The more water soluble compounds (> 2% solubility) and compounds with boiling points above 200°C are purged from the water matrix with lower efficiencies. These analytes may be more susceptible to matrix effects.

## 2. SUMMARY OF METHOD

2.1 Volatile organic compounds and surrogates with low water solubility are extracted (purged) from the sample matrix by bubbling an inert gas through the aqueous sample. Purged sample components are trapped in a tube containing suitable sorbent materials. When purging is complete, the sorbent tube is heated and backflushed with helium to desorb the trapped sample components into a capillary gas chromatography (GC) column interfaced to a mass spectrometer (MS). The column is temperature programmed to facilitate the separation of the method analytes which are then detected with the MS. Compounds eluting from the GC column are identified by comparing their measured mass spectra and retention times to reference spectra and retention times in a data base. Reference spectra and retention times for analytes are obtained by the measurement of calibration standards under the same conditions used for samples. The concentration of each identified component is measured by relating the MS response of the quantitation ion produced by that compound to the MS response of the quantitation ion produced by a compound that is used as an internal standard. Surrogate analytes, whose concentrations are known in every sample, are measured with the same internal standard calibration procedure.

## 3. DEFINITIONS

3.1 INTERNAL STANDARD (IS) -- A pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same sample or solution. The internal standard must be an analyte that is not a sample component.

3.2 SURROGATE ANALYTE (SA) -- A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction or other processing and is measured with the same procedures used to measure other sample components. The purpose of the SA is to monitor method performance with each sample.

3.3 LABORATORY DUPLICATES (LD1 and LD2) -- Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicates precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 FIELD DUPLICATES (FD1 and FD2) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.5 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 FIELD REAGENT BLANK (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.7 LABORATORY PERFORMANCE CHECK SOLUTION (LPC) -- A solution of one or more compounds (analytes, surrogates, internal standard, or other test compounds) used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

3.8 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.9 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.10 STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.11 PRIMARY DILUTION STANDARD SOLUTION (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.12 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.13 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

## 4. INTERFERENCES

4.1 During analysis, major contaminant sources are volatile materials in the laboratory and impurities in the inert purging gas and in the sorbent trap. The use of Teflon tubing, Teflon thread sealants, or flow controllers with rubber components in the purging device should be avoided since such materials out-gas organic compounds which will be concentrated in the trap during the purge operation. Analyses of laboratory reagent blanks provide information about the presence of contaminants. When potential interfering peaks are noted in laboratory reagent blanks, the analyst should change the purge gas source and regenerate the molecular sieve purge gas filter. Subtracting blank values from sample results is not permitted.

4.2 Interfering contamination may occur when a sample containing low concentrations of volatile organic compounds is analyzed immediately after a sample containing relatively high concentrations of volatile organic compounds. A preventive technique is between-sample rinsing of the purging apparatus and sample syringes with two portions of reagent water. After analysis of a sample containing high concentrations of volatile organic compounds, one or more laboratory reagent blanks should be analyzed to check for cross-contamination.

4.3 Special precautions must be taken to determine methylene chloride. The analytical and sample storage area should be isolated from all atmospheric sources of methylene chloride, otherwise random background levels will result. Since methylene chloride will permeate Teflon tubing, all GC carrier gas lines and purge gas plumbing should be constructed of stainless steel or copper tubing. Laboratory worker's clothing should be cleaned frequently since clothing previously exposed to methylene chloride fumes during common liquid/liquid extraction procedures can contribute to sample contamination.

4.4 Traces of ketones, methylene chloride, and some other organic solvents can be present even in the highest purity methanol. This is another potential source of contamination, and should be assessed before standards are prepared in the methanol.

## 5. SAFETY

5.1 The toxicity or carcinogenicity of chemicals used in this method has not been precisely defined; each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining awareness of OSHA regulations regarding safe handling of chemicals used in this method. Additional references to laboratory safety are available (4-6) for the information of the analyst.

5.2 The following method analytes have been tentatively classified as known or suspected human or mammalian carcinogens: benzene, carbon tetrachloride, 1,4-dichlorobenzene, 1,2-dichlorethane, hexachlorobutadiene, 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane, chloroform, 1,2-dibromoethane,tetrachloroethene, trichloroethene, and vinyl chloride. Pure standard materials and stock standard solutions of these compounds should be handled in a hood. A NIOSH/MESA approved toxic gas respirator should be worn when the analyst handles high concentrations of these toxic compounds.

## 6. EQUIPMENT AND SUPPLIES

6.1 SAMPLE CONTAINERS -- 40-mL to 120-mL screw cap vials each equipped with a Teflon faced silicone septum. Prior to use, wash vials and septa with detergent and rinse with tap and distilled water. Allow the vials and septa to air dry at room temperature, place in a 105°C oven for 1 hr, then remove and allow to cool in an area known to be free of organics.

6.2 PURGE AND TRAP SYSTEM -- The purge and trap system consists of three separate pieces of equipment: purging device, trap, and desorber. Systems are commercially available from several sources that meet all of the following specifications.

6.2.1 The all glass purging device (Figure 1) should be designed to accept 25-mL samples with a water column at least 5 cm deep. A smaller (5-mL) purging device is recommended if the GC/MS system has adequate sensitivity to obtain the method detection limits required. Gaseous volumes above the sample must be kept to a minimum (< 15 mL) to eliminate dead volume effects. A glass frit should be installed at the base of the sample chamber so the purge gas passes through the water column as finely divided bubbles with a diameter of < 3 mm at the origin. Needle spargers may be used, however, the purge gas must be introduced at a point about 5 mm from the base of the water column. The use of a moisture control device is recommended to prohibit much of the trapped water vapor from entering the GC/MS and eventually causing instrumental problems.

6.2.2 The trap (Figure 2) must be at least 25 cm long and have an inside diameter of at least 0.105 in. Starting from the inlet, the trap should contain 1.0 cm of methyl silicone coated packing and the following amounts of adsorbents: 1/3 of 2,6-diphenylene oxide polymer, 1/3 of silica gel, and 1/3 of coconut charcoal. If it is not necessary to determine dichlorodifluoromethane, the charcoal can be eliminated and the polymer increased to fill 2/3 of the trap. Before initial use, the trap should be conditioned overnight at 180°C by backflushing with an inert gas flow of at least 20 mL/min. Vent the trap effluent to the room, not to the analytical column. Prior to daily use, the trap should be conditioned for 10 min at 180°C with backflushing. The trap may be vented to the analytical column during daily conditioning; however, the column must be run through the temperature program prior to analysis of samples. The use of alternative sorbents is acceptable, depending on the particular set of target analytes or other problems encountered, but the new trap packing must meet all quality control criteria described in Sect. 9.

6.2.3 The use of the methyl silicone coated packing is recommended, but not mandatory. The packing serves a dual purpose of protecting the Tenax adsorbant from aerosols, and also of insuring that the Tenax is fully enclosed within the heated zone of the trap thus eliminating potential cold spots. Alternatively, silanized glass wool may be used as a spacer at the trap inlet.

6.2.4 The desorber (Figure 2) must be capable of rapidly heating the trap to 180°C either prior to or at the beginning of the flow of desorption gas. The polymer section of the trap should not be heated higher than 200°C or the life expectancy of the trap will decrease. Trap failure is characterized by a pressure drop in excess of 3 $lb/in^2$ across the trap during purging or by poor bromoform sensitivities. The desorber design illustrated in Fig. 2 meets these criteria.

## 6.3 GAS CHROMATOGRAPHY/MASS SPECTROMETER/DATA SYSTEM (GC/MS/DS)

6.3.1 The GC must be capable of temperature programming and should be equipped with variable-constant differential flow controllers so that the column flow rate will remain constant throughout desorption and temperature program operation. If the column oven is to be cooled to 10°C or lower, a subambient oven controller will likely be required. If syringe injections of 4-bromofluorobenzene (BFB) will be used, a split/splitless injection port is required.

6.3.2 Capillary GC Columns. Any gas chromatography column that meets the performance specifications of this method may be used (Sect. 10.2.4.1). Separations of the calibration mixture must be equivalent or better than those described in this method. Four useful columns have been evaluated, and observed compound retention times for these columns are listed in Table 2.

6.3.2.1 Column 1 -- 60 m x 0.75 mm ID VOCOL (Supelco, Inc.) glass wide-bore capillary with a 1.5 μm film thickness.

Column 2 -- 30 m x 0.53 mm ID DB-624 (J&W Scientific, Inc.) fused silica capillary with a 3 μm film thickness.

Column 3 -- 30 m x 0.32 mm ID DB-5 (J&W Scientific, Inc.) fused silica capillary with a 1 μm film thickness.

Column 4 -- 75 m x 0.53 mm id DB-624 (J&W Scientific, Inc.) fused silica capillary with a 3 μm film thickness.

6.3.3 Interfaces between the GC and MS. The interface used depends on the column selected and the gas flow rate.

6.3.3.1 The wide-bore columns 1, 2, and 4 have the capacity to accept the standard gas flows from the trap during thermal desorption, and chromatography can begin with the onset of thermal desorption. Depending on the pumping capacity of the MS, an additional interface between the end of the column and the MS may be required. An open split interface (7) or an all-glass jet separator is an acceptable interface. Any interface can be used if the performance specifications described in this method (Sect. 9 and 10) can be achieved. The end of the transfer line after the interface, or the end of the analytical column if no interface is used, should be placed within a few mm of the MS ion source.

6.3.3.2 When narrow bore column 3 is used, a cryogenic interface placed just in front of the column inlet is suggested. This interface condenses the desorbed sample components in a narrow band on an uncoated fused silica precolumn using liquid nitrogen cooling. When all analytes have been desorbed from the trap, the interface is rapidly heated to transfer them to the analytical column. The end of the analytical column should be placed within a few mm of the MS ion source. A potential problem with this interface is blockage of the interface by frozen water from the trap. This condition will result in a major loss in sensitivity and chromatographic resolution.

6.3.4 The mass spectrometer must be capable of electron ionization at a nominal electron energy of 70 eV. The spectrometer must be capable of scanning from 35 to 260 amu with a complete scan cycle time (including scan overhead) of 2 sec or less. (Scan cycle time = Total MS data acquisition time in seconds divided by number of scans in the chromatogram.) The spectrometer must produce a mass spectrum that meets all criteria in Table 3 when 25 ng or less of 4-bromofluorobenzene (BFB) is introduced into the GC. An average spectrum across the BFB GC peak may be used to test instrument performance.

6.3.5 An interfaced data system is required to acquire, store, reduce, and output mass spectral data. The computer software should have the capability of processing stored GC/MS data by recognizing a GC peak within any given retention time window, comparing the mass spectra from the GC peak with spectral data in a user-created data base, and generating a list of tentatively identified compounds with their retention times and scan numbers. The software must allow integration of the ion abundance of any specific ion between specified time or scan number limits. The software should also allow calculation of response factors as defined in Sect. 10.2.6 (or construction of a linear or second order regression calibration curve), calculation of response factor statistics (mean and standard deviation), and calculation of concentrations of analytes using either the calibration curve or the equation in Sect. 12.

### 6.4 SYRINGE AND SYRINGE VALVES

6.4.1 Two 5-mL or 25-mL glass hypodermic syringes with Luer-Lok tip (depending on sample volume used).

6.4.2 Three 2-way syringe valves with Luer ends.

6.4.3 Micro syringes - 10, 100 µL.

6.4.4 Syringes - 0.5, 1.0, and 5-mL, gas tight with shut-off valve.

### 6.5 MISCELLANEOUS

6.5.1 Standard solution storage containers -- 15-mL bottles with Teflon lined screw caps.

## 7. REAGENTS AND STANDARDS

### 7.1 TRAP PACKING MATERIALS

7.1.1 2,6-Diphenylene oxide polymer, 60/80 mesh, chromatographic grade (Tenax GC or equivalent).

7.1.2 Methyl silicone packing (optional) -- OV-1 (3%) on Chromosorb W, 60/80 mesh, or equivalent.

7.1.3 Silica gel -- 35/60 mesh, Davison, grade 15 or equivalent.

7.1.4 Coconut charcoal -- Prepare from Barnebey Cheney, CA-580-26 lot #M-2649 by crushing through 26 mesh screen.

### 7.2 REAGENTS

7.2.1 Methanol -- Demonstrated to be free of analytes.

7.2.2 Reagent water -- Prepare reagent water by passing tap water through a filter bed containing about 0.5 kg of activated carbon, by using a water purification system, or by boiling distilled water for 15 min followed by a 1-h purge with inert gas while the water temperature is held at 90°C. Store in clean, narrow-mouth bottles with Teflon lined septa and screw caps.

7.2.3 Hydrochloric acid (1+1) -- Carefully add measured volume of conc. HCl to equal volume of reagent water.

7.2.4 Vinyl chloride -- Certified mixtures of vinyl chloride in nitrogen and pure vinyl chloride are available from several sources (for example, Matheson, Ideal Gas Products, and Scott Gases).

7.2.5 Ascorbic acid -- ACS reagent grade, granular.

7.2.6 Sodium thiosulfate -- ACS reagent grade, granular.

7.3 STOCK STANDARD SOLUTIONS -- These solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedures. One of these solutions is required for every analyte of concern, every surrogate, and the internal standard. A useful working concentration is about 1-5 mg/mL.

7.3.1 Place about 9.8 mL of methanol into a 10-mL ground-glass stoppered volumetric flask. Allow the flask to stand, unstoppered, for about 10 min or until all alcohol-wetted surfaces have dried and weigh to the nearest 0.1 mg.

7.3.2 If the analyte is a liquid at room temperature, use a 100-µL syringe and immediately add two or more drops of reference standard to the flask. Be sure that the reference standard falls directly into the alcohol without contacting the neck of the flask. If the analyte is a gas at room temperature, fill a 5-mL valved gas-tight syringe with the standard to the 5.0-mL mark, lower the needle to 5 mm above the methanol meniscus, and slowly inject the standard into the neck area of the flask. The gas will rapidly dissolve in the methanol.

7.3.3 Reweigh, dilute to volume, stopper, then mix by inverting the flask several times. Calculate the concentration in µg/µL from the net gain in weight. When compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard.

7.3.4 Store stock standard solutions in 15-mL bottles equipped with Teflon lined screw caps. Methanol solutions of acrylonitrile, methyl iodide, and methyl acrylate are stable for only one week at 4°C. Methanol solutions prepared from other liquid analytes are stable for at least 4 weeks when stored at 4°C. Methanol solutions prepared from gaseous analytes are not stable for more than 1 week when stored at < 0°C; at room temperature, they must be discarded after 1 day.

7.4 PRIMARY DILUTION STANDARDS -- Use stock standard solutions to prepare primary dilution standard solutions that contain all the analytes of concern in methanol or other suitable solvent. The primary dilution standards should be prepared at concentrations that can be easily diluted to prepare aqueous calibration solutions that will bracket the working concentration range. Store the primary dilution standard solutions with minimal headspace and check frequently for signs of deterioration or evaporation, especially just before preparing calibration solutions. Storage times described for stock standard solutions in Sect. 7.3.4 also apply to primary dilution standard solutions.

7.5 FORTIFICATION SOLUTIONS FOR INTERNAL STANDARD AND SURROGATES

7.5.1 A solution containing the internal standard and the surrogate compounds is required to prepare laboratory reagent blanks (also used as a laboratory performance check solution), and to fortify each sample. Prepare a fortification solution containing fluorobenzene (internal standard), 1,2- dichlorobenzene-$d_4$ (surrogate), and BFB (surrogate) in methanol at concentrations of 5 µg/mL of each (any appropriate concentration is acceptable). A 5-µL aliquot of this solution added to a 25-mL water sample volume gives concentrations of 1 µg/L of each. A 5-µL aliquot of this solution added to a 5-mL water sample volume gives a concentration of 5 µg/L of each. Additional internal standards and surrogate analytes are optional. Additional surrogate compounds should be similar in physical and chemical characteristics to the analytes of concern.

7.6 PREPARATION OF LABORATORY REAGENT BLANK (LRB) -- Fill a 25-mL (or 5-mL) syringe with reagent water and adjust to the mark (no air bubbles). Inject an appropriate volume of the fortification solution containing the internal standard and surrogates through the Luer Lok valve into the reagent water. Transfer the LRB to the purging device. See Sect. 11.1.2.

7.7 PREPARATION OF LABORATORY FORTIFIED BLANK -- Prepare this exactly like a calibration standard (Sect. 7.8). This is a calibration standard that is treated as a sample.

### 7.8 PREPARATION OF CALIBRATION STANDARDS

7.8.1 The number of calibration solutions (CALs) needed depends on the calibration range desired. A minimum of three CAL solutions is required to calibrate a range of a factor of 20 in concentration. For a factor of 50, use at least four standards, and for a factor of 100 at least five standards. One calibration standard should contain each analyte of concern at a concentration of 2-10 times the method detection limit (Tables 4, 5, and 7) for that compound. The other CAL standards should contain each analyte of concern at concentrations that define the range of the method. Every CAL solution contains the internal standard and the surrogate compounds at the same concentration (5 µg/L suggested for a 5-mL sample; 1 µg/L for a 25-mL sample).

7.8.2 To prepare a calibration standard, add an appropriate volume of a primary dilution standard containing all analytes of concern to an aliquot of acidified (pH 2) reagent water in a volumetric flask. Also add an appropriate volume of internal standard and surrogate compound solution from Sect. 7.5.1. Use a microsyringe and rapidly inject the methanol solutions into the expanded area of the filled volumetric flask. Remove the needle as quickly as possible after injection. Mix by inverting the flask three times only. Discard the contents contained in the neck of the flask. Aqueous standards are not stable in a volumetric flask and should be discarded after 1 hr unless transferred to a sample bottle and sealed immediately.

## 8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

### 8.1 SAMPLE COLLECTION, DECHLORINATION, AND PRESERVATION

8.1.1 Collect all samples in duplicate. If samples, such as finished drinking water or waste water, are suspected to contain residual chlorine, add about 25 mg of ascorbic acid per 40 mL of sample to the sample bottle before filling. If the residual chlorine is likely to be present > 5 mg/L, a determination of the amount of the chlorine may be necessary. Diethyl-p-phenylenediamine (DPD) test kits are commercially available to determine residual chlorine in the field. Add an additional 25 mg of ascorbic acid per each 5 mg/L of residual chlorine. If compounds boiling below 25°C are not to be determined, sodium thiosulfate may be used to reduce the residual chlorine. Fill sample bottles to overflowing, but take care not to flush out the rapidly dissolving ascorbic acid. No air bubbles should pass through the sample as the bottle is filled, or be trapped in the sample when the bottle is sealed. Adjust the pH of the duplicate samples to

< 2 by carefully adding two drops of 1:1 HCl for each 40 mL of sample. Seal the sample bottles, Teflon face down, and shake vigorously for 1 min. Do not mix the ascorbic acid or sodium thiosulfate with the HCl prior to sampling.

8.1.2 When sampling from a water tap, open the tap and allow the system to flush until the water temperature has stabilized (usually about 10 min). Adjust the flow to about 500 mL/min and collect duplicate samples from the flowing stream.

8.1.3 When sampling from an open body of water, such as surface water, waste water, and possible leachate samples, partially fill a 1-quart wide-mouth bottle or 1-L beaker with sample from a representative area. Fill a 60 mL or a 120 mL sample vial with sample from the larger container, and adjust the pH of the sample to about 2 by adding 1+1 HCl dropwise while stirring. Check the pH with narrow range (1.4 to 2.8) pH paper. Record the number of drops of acid necessary to adjust the pH to 2. To collect actual samples, refill the large container with fresh sample and pour sample into sample vials. Follow filling instructions in Sect. 8.1.1. Add the appropriate number of drops of 1+1 HCl to each sample to adjust the pH to about 2. If samples are suspected to contain residual chlorine, add ascorbic acid or sodium thiosulfate according to Sect. 8.1.1.

8.1.4 The samples must be chilled to about 4°C when collected and maintained at that temperature until analysis. Field samples that will not be received at the laboratory on the day of collection must be packaged for shipment with sufficient ice to ensure that they will arrive at the laboratory with a substantial amount of ice remaining in the cooler.

8.1.5 If a sample foams vigorously when HCl is added, discard that sample. Collect a set of duplicate samples but do not acidify them. These samples must be flagged as "not acidified" and must be stored at 4°C or below. These samples must be analyzed within 24 hr of collection time.

## 8.2 SAMPLE STORAGE

8.2.1 Store samples at $\leq$ 4°C until analysis. The sample storage area must be free of organic solvent vapors and direct or intense light.

8.2.2 Analyze all samples within 14 days of collection. Samples not analyzed within this period must be discarded and replaced.

## 8.3 FIELD REAGENT BLANKS (FRB)

8.3.1 Duplicate FRBs must be handled along with each sample set, which is composed of the samples collected from the same general sample site at approximately the same time. At the laboratory, fill field blank sample bottles with reagent water and sample preservatives, seal, and ship to the sampling site along with empty sample bottles and back to the laboratory with filled sample bottles. Wherever a set of samples is shipped and stored, it is accompanied by appropriate blanks. FRBs must remain hermetically sealed until analysis.

8.3.2 Use the same procedures used for samples to add ascorbic acid and HCl to blanks (Sect. 8.1.1). The same batch of ascorbic acid and HCl should be used for the field reagent blanks in the field.

## 9. QUALITY CONTROL

9.1 Quality control (QC) requirements are the initial demonstration of laboratory capability followed by regular analyses of laboratory reagent blanks, field reagent blanks, and laboratory fortified blanks. Each laboratory must maintain records to document the quality of the data generated. Additional quality control practices are recommended.

9.2 Initial demonstration of low system background. Before any samples are analyzed, it must be demonstrated that a laboratory reagent blank (LRB) is reasonably free of contamination that would prevent the determination of any analyte of concern. Sources of background contamination are glassware, purge gas, sorbents, and equipment. Background contamination must be reduced to an acceptable level before proceeding with the next section. In general, background from method analytes should be below the method detection limit.

9.3 Initial demonstration of laboratory accuracy and precision. Analyze five to seven replicates of a laboratory fortified blank containing each analyte of concern at a concentration in the range of 0.2-5 μg/L (see appropriate regulations and maximum contaminant levels for guidance on appropriate concentrations).

9.3.1 Prepare each replicate by adding an appropriate aliquot of a quality control sample to reagent water. If a quality control sample containing the method analytes is not available, a primary dilution standard made from a source of reagents different than those used to prepare the calibration standards may be used. Also add the appropriate amounts of internal standard and surrogate compounds. Analyze each replicate according to the procedures described in Sect. 11, and on a schedule that results in the analyses of all replicates over a period of several days.

9.3.2 Calculate the measured concentration of each analyte in each replicate, the mean concentration of each analyte in all replicates, and mean accuracy (as mean percentage of true value) for each analyte, and the precision (as relative standard deviation, RSD) of the measurements for each analyte. Calculate the MDL of each analyte using the equation described in Sect. 13.2 (3).

9.3.3 For each analyte, the mean accuracy, expressed as a percentage of the true value, should be 80-120% and the RSD should be < 20%. Some analytes, particularly the early eluting gases and late eluting higher molecular weight compounds, are measured with less accuracy and precision than other analytes. The MDLs must be sufficient to detect analytes at the required levels according to the SDWA Regulations. If these criteria are not met for an analyte, take remedial action and repeat the measurements for that analyte to demonstrate acceptable performance before samples are analyzed.

9.3.4 Develop and maintain a system of control charts to plot the precision and accuracy of analyte and surrogate measurements as a function of time. Charting surrogate recoveries is an especially valuable activity because surrogates are present in every sample and the analytical results will form a significant record of data quality.

9.4 Monitor the integrated areas of the quantitation ions of the internal standards and surrogates (Table 1) in all samples, continuing calibration checks, and blanks. These should remain reasonably constant over time. An abrupt change may indicate a matrix effect or an instrument problem. If a cryogenic interface is utilized, it may indicate an inefficient transfer from the trap to the column. These samples must be reanalyzed or a laboratory fortified duplicate sample analyzed to test for matrix effect. A more gradual drift of more than 50% in any area is indicative of a loss in sensitivity, and the problem must be found and corrected.

9.5 LABORATORY REAGENT BLANKS (LRB) -- With each batch of samples processed as a group within a work shift, analyze a LRB to determine the background system contamination. A FRB (Sect. 9.7) may be used in place of a LRB.

9.6 With each batch of samples processed as a group within a work shift, analyze a single laboratory fortified blank (LFB) containing each analyte of concern at a concentration as determined in Sect. 9.3. If more than 20 samples are included in a batch, analyze one LFB for every 20 samples. Use the procedures described in Sect. 9.3.3 to evaluate the accuracy of the measurements, and to estimate whether the MDLs can be obtained. If acceptable accuracy and MDLs cannot be achieved, the problem must be located and corrected before further samples are analyzed. Add these results to the ongoing control charts to document data quality.

9.7 With each set of field samples a field reagent blank (FRB) should be analyzed. The results of these analyses will help define contamination resulting from field sampling and transportation activities. If the FRB shows unacceptable contamination, a LRB must be measured to define the source of the impurities.

9.8 At least quarterly, replicate LFBs should be analyzed to determine the precision of the laboratory measurements. Add these results to the ongoing control charts to document data quality.

9.9 At least quarterly, analyze a quality control sample (QCS) from an external source. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem source.

9.10 Sample matrix effects have not been observed when this method is used with distilled water, reagent water, drinking water, or ground water. Therefore, analysis of a laboratory fortified sample matrix (LFM) is not required unless the criteria in Section 9.4 are not met. If matrix effects are observed or suspected to be causing low recoveries, analyze a laboratory fortified matrix sample for that matrix. The sample results should be flagged and the LFM results should be reported with them.

9.11 Numerous other quality control measures are incorporated into other parts of this procedure, and serve to alert the analyst to potential problems.

## 10. CALIBRATION AND STANDARDIZATION

10.1 Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed and is required intermittently throughout sample analysis as dictated by results of continuing calibration checks. After initial calibration is successful, a continuing calibration check is required at the beginning of each 8 hr. period during which analyses are performed. Additional periodic calibration checks are good laboratory practice.

### 10.2 INITIAL CALIBRATION

10.2.1 Calibrate the mass and abundance scales of the MS with calibration compounds and procedures prescribed by the manufacturer with any modifications necessary to meet the requirements in Sect. 10.2.2.

10.2.2 Introduce into the GC (either by purging a laboratory reagent blank or making a syringe injection) 25 ng or less of BFB and acquire mass spectra for m/z 35-260 at 70 eV (nominal). Use the purging procedure and/or GC conditions given in Sect. 11. If the spectrum does not meet all criteria in Table 3, the MS must be retuned and adjusted to meet all criteria before proceeding with calibration. An average spectrum across the GC peak may be used to evaluate the performance of the system.

10.2.3 Purge a medium CAL solution, (e.g., 10-20 $\mu$g/L) using the procedure given in Sect. 11.

10.2.4 Performance criteria for the medium calibration. Examine the stored GC/MS data with the data system software. Figures 3 and 4 shown acceptable total ion chromatograms.

10.2.4.1 GC performance. Good column performance will produce symmetrical peaks with minimum tailing for most compounds. If peaks are unusually broad, or if peaks are running together with little vallies between them, the wrong column has been selected or remedial action is probably necessary (Sect.10.3.6).

10.2.4.2 MS sensitivity. The GC/MS/DS peak identification software should be able to recognize a GC peak in the appropriate retention time window for each of the compounds in calibration solution, and make correct tentative identifications. If fewer than 99% of the compounds are recognized, system maintenance is required. See Sect. 10.3.6.

10.2.5 If all performance criteria are met, purge an aliquot of each of the other CAL solutions using the same GC/MS conditions.

10.2.6 Calculate a response factor (RF) for each analyte and isomer pair for each CAL solution using the internal standard fluorobenzene. Table 1 contains suggested quantitation ions for all compounds. This calculation is supported in acceptable GC/MS data system software (Sect. 6.3.5), and many other software programs. RF is a unitless number, but units used to express quantities of analyte and internal standard must be equivalent.

$$RF = \frac{(A_x)(Q_{is})}{(A_{is})(Q_x)}$$

where: $A_x$ = integrated abundance of the quantitation ion of the analyte.
$A_{is}$ = integrated abundance of the quantitation ion of the internal standard.
$Q_x$ = quantity of analyte purged in nanograms or concentration units.
$Q_{is}$ = quantity of internal standard purged in ng or concentration units.

10.2.6.1 For each analyte and surrogate, calculate the mean RF from analyses of CAL solutions. Calculate the standard deviation (SD) and the relative standard deviation (RSD) from each mean: RSD = 100 (SD/M). If the RSD of any analyte or surrogate mean RF exceeds 20%, either analyze additional aliquots of appropriate CAL solutions to obtain an acceptable RSD of RFs over the entire concentration range, or take action to improve GC/MS performance Sect. 10.3.6). Surrogate compounds are present at the same concentration on every sample, calibration standard, and all types of blanks.

10.2.7 As an alternative to calculating mean response factors and applying the RSD test, use the GC/MS data system software or other available software to generate a linear or second order regression calibration curve

10.3 CONTINUING CALIBRATION CHECK -- Verify the MS tune and initial calibration at the beginning of each 8-hr work shift during which analyses are performed using the following procedure.

10.3.1 Introduce into the GC (either by purging a laboratory reagent blank or making a syringe injection) 25 ng or less of BFB and acquire a mass spectrum that includes data for m/z 35-260. If the spectrum does not meet all criteria (Table 3), the MS must be retuned and adjusted to meet all criteria before proceeding with the continuing calibration check.

10.3.2 Purge a medium concentration CAL solution and analyze with the same conditions used during the initial calibration.

10.3.3 Demonstrate acceptable performance for the criteria shown in Sect. 10.2.4.

10.3.4 Determine that the absolute areas of the quantitation ions of the internal standard and surrogates have not decreased by more than 30% from the areas measured in the most recent continuing calibration check, or by more than 50% from the areas measured during initial calibration. If these areas have decreased by more than these amounts, adjustments must be made to restore system sensitivity. These adjustments may require cleaning of the MS ion source, or other maintenance as indicated in Sect. 10.3.6, and recalibration. Control charts are useful aids in documenting system sensitivity changes.

10.3.5 Calculate the RF for each analyte of concern and surrogate compound from the data measured in the continuing calibration check. The RF for each analyte and surrogate must be within 30% of the mean value measured in the initial calibration. Alternatively, if a linear or second order regression is used, the concentration measured using the calibration curve must be within 30% of the true value of the concentration in the medium calibration solution. If these conditions do not exist, remedial action must be taken which may require re-calibration.

10.3.6 Some possible remedial actions. Major maintenance such as cleaning an ion source, cleaning quadrupole rods, etc. require returning to the initial calibration step.

10.3.6.1 Check and adjust GC and/or MS operating conditions; check the MS resolution, and calibrate the mass scale.

10.3.6.2 Clean or replace the splitless injection liner; silanize a new injection liner. This applies only if the injection liner is an integral part of the system.

10.3.6.3 Flush the GC column with solvent according to manufacturer's instructions.

10.3.6.4 Break off a short portion (about 1 meter) of the column from the end near the injector; or replace GC column. This action will cause a slight change in retention times. Analyst may need to redefine retention windows.

10.3.6.5 Prepare fresh CAL solutions, and repeat the initial calibration step.

10.3.6.6 Clean the MS ion source and rods (if a quadrupole).

10.3.6.7 Replace any components that allow analytes to come into contact with hot metal surfaces.

10.3.6.8 Replace the MS electron multiplier, or any other faulty components.

10.3.6.9 Replace the trap, especially when only a few compounds fail the criteria in Sect. 10.3.5 while the majority are determined successfully. Also check for gas leaks in the purge and trap unit as well as the rest of the analytical system.

10.4 Optional calibration for vinyl chloride using a certified gaseous mixture of vinyl chloride in nitrogen can be accomplished by the following steps.

10.4.1 Fill the purging device with 25.0 mL (or 5-mL) of reagent water or aqueous calibration standard.

10.4.2 Start to purge the aqueous mixture. Inject a known volume (between 100 and 2000 µL) of the calibration gas (at room temperature) directly into the purging device with a gas tight syringe. Slowly inject the gaseous sample through a septum seal at the top of the purging device at 2000 µL/min. If the injection of the standard is made through the aqueous sample inlet port, flush the dead volume with several mL of room air or carrier gas. Inject the gaseous standard before 5 min of the 11-min purge time have elapsed.

10.4.3 Determine the aqueous equivalent concentration of vinyl chloride standard, in μg/L, injected with the equation:

$$S = 0.102\ (C)(V)$$

where S = Aqueous equivalent concentration of vinyl chloride standard in μg/L;
C = Concentration of gaseous standard in mg/L (v/v);
V = Volume of standard injected in mL.

## 11. PROCEDURE

### 11.1 SAMPLE INTRODUCTION AND PURGING

11.1.1 This method is designed for a 25-mL sample volume, but a smaller (5 mL) sample volume is recommended if the GC/MS system has adequate sensitivity to achieve the required method detection limits. Adjust the helium purge gas flow rate to 40 mL/min. Attach the trap inlet to the purging device and open the syringe valve on the purging device.

11.1.2 Remove the plungers from two 25-mL (or 5-mL depending on sample size) syringes and attach a closed syringe valve to each. Warm the sample to room temperature, open the sample bottle, and carefully pour the sample into one of the syringe barrels to just short of overflowing. Replace the syringe plunger, invert the syringe, and compress the sample. Open the syringe valve and vent any residual air while adjusting the sample volume to 25.0-mL (or 5-mL). To all samples, blanks, and calibration standards, add 5-μL (or an appropriate volume) of the fortification solution containing the internal standard and the surrogates to the sample through the syringe valve. Close the valve. Fill the second syringe in an identical manner from the same sample bottle. Reserve this second syringe for a reanalysis if necessary.

11.1.3 Attach the sample syringe valve to the syringe valve on the purging device. Be sure that the trap is cooler than 25°C, then open the sample syringe valve and inject the sample into the purging chamber. Close both valves and initiate purging. Purge the sample for 11.0 min at ambient temperature.

11.1.4 Standards and samples must be analyzed in exactly the same manner. Room temperature changes in excess of 10°F may adversely affect the accuracy and precision of the method.

## 11.2 SAMPLE DESORPTION

11.2.1 Non-cryogenic interface -- After the 11-min purge, place the purge and trap system in the desorb mode and preheat the trap to 180°C without a flow of desorption gas. Then simultaneously start the flow of desorption gas at a flow rate suitable for the column being used (optimum desorb flow rate is 15 mL/min) for about 4 min, begin the GC temperature program, and start data acquisition.

11.2.2 Cryogenic interface -- After the 11-min purge, place the purge and trap system in the desorb mode, make sure the cryogenic interface is a -150°C or lower, and rapidly heat the trap to 180°C while backflushing with an inert gas at 4 mL/min for about 5 min. At the end of the 5 min desorption cycle, rapidly heat the cryogenic trap to 250°C, and simultaneously begin the temperature program of the gas chromatograph, and start data acquisition.

11.2.3 While the trapped components are being introduced into the gas chromatograph (or cryogenic interface), empty the purging device using the sample syringe and wash the chamber with two 25-mL flushes of reagent water. After the purging device has been emptied, leave syringe valve open to allow the purge gas to vent through the sample introduction needle.

11.3 GAS CHROMATOGRAPHY/MASS SPECTROMETRY -- Acquire and store data over the nominal mass range 35-260 with a total cycle time (including scan overhead time) of 2 sec or less. If water, methanol, or carbon dioxide cause a background problem, start at 47 or 48 m/z. If ketones are to be determined, data must be acquired starting at m/z 43. Cycle time must be adjusted to measure five or more spectra during the elution of each GC peak. Suggested temperature programs are provided below. Alternative temperature programs can be used.

11.3.1 Single ramp linear temperature program for wide bore column 1 and 2 with a jet separator. Adjust the helium carrier gas flow rate to within the capacity of the separator, or about 15 mL/min. The column temperature is reduced 10°C and held for 5 min from the beginning of desorption, then programmed to 160°C at 6°C/min, and held until all components have eluted.

11.3.2 Multi-ramp temperature program for wide bore column 2 with the open split interface. Adjust the helium carrier gas flow rate to about 4.6 mL/min. The column temperature is reduced to 10°C and held for 6 min from the beginning of desorption, then heated to 70°C at 10°/min, heated to 120°C at 5°/min, heated to 180° at 8°/min, and held at 180° until all compounds have eluted.

11.3.3 Single ramp linear temperature program for narrow bore column 3 with a cryogenic interface. Adjust the helium carrier gas flow rate to about 4 mL/min. The column temperature is reduced to 10°C and held for 5 min from the beginning of vaporization from the cryogenic trap, programmed at 6°/min for 10 min, then 15°/min for 5 min to 145°C, and held until all components have eluted.

11.3.4 Multi-ramp temperature program for wide bore column 4 with the open split interface. Adjust the helium carrier gas flow rate to about 7.0 mL/min. The column temperature is - 10°C and held for 6 min. from beginning of desorption, then heated to 100°C at 10°C/min, heated to 200°C at 5°C/min and held at 200°C for 8 min or until all compounds of interest had eluted.

11.4 TRAP RECONDITIONING -- After desorbing the sample for 4 min, recondition the trap by returning the purge and trap system to the purge mode. Wait 15 sec, then close the syringe valve on the purging device to begin gas flow through the trap. Maintain the trap temperature at 180°C. Maintain the moisture control module, if utilized, at 90°C to remove residual water. After approximately 7 min, turn off the trap heater and open the syringe valve to stop the gas flow through the trap. When the trap is cool, the next sample can be analyzed.

11.5 TERMINATION OF DATA ACQUISITION -- When all the sample components have eluted from the GC, terminate MS data acquisition. Use appropriate data output software to display full range mass spectra and appropriate plots of ion abundance as a function of time. If any ion abundance exceeds the system working range, dilute the sample aliquot in the second syringe with reagent water and analyze the diluted aliquot.

11.6 IDENTIFICATION OF ANALYTES -- Identify a sample component by comparison of its mass spectrum (after background subtraction) to a reference spectrum in the user-created data base. The GC retention time of the sample component should be within three standard deviations of the mean retention time of the compound in the calibration mixture.

11.6.1 In general, all ions that are present above 10% relative abundance in the mass spectrum of the standard should be present in the mass spectrum of the sample component and should agree within absolute 20%. For example, if an ion has a relative abundance of 30% in the standard spectrum, its abundance in the sample spectrum should be in the range of 10 to 50%. Some ions, particularly the molecular ion, are of special importance, and should be evaluated even if they are below 10% relative abundance.

11.6.2 Identification requires expert judgment when sample components are not resolved chromatographically and produce mass spectra containing ions contributed by more than one analyte.

When GC peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima), appropriate analyte spectra and background spectra can be selected by examining plots of characteristic ions for tentatively identified components. When analytes coelute (i.e., only one GC peak is apparent), the identification criteria can be met but each analyte spectrum will contain extraneous ions contributed by the coeluting compound. Because purgeable organic compounds are relatively small molecules and produce comparatively simple mass spectra, this is not a significant problem for most method analytes.

11.6.3 Structural isomers that produce very similar mass spectra can be explicitly identified only if they have sufficiently different GC retention times. Acceptable resolution is achieved if the height of the valley between two peaks is less than 25% of the average height of the two peaks. Otherwise, structural isomers are identified as isomeric pairs. Two of the three isomeric xylenes and two of the three dichlorobenzenes are examples of structural isomers that may not be resolved on the capillary columns. If unresolved, these groups of isomers must be reported as isomeric pairs.

11.6.4 Methylene chloride, acetone, carbon disulfide, and other background components appear in variable quantities in laboratory and field reagent blanks, and generally cannot be accurately measured. Subtraction of the concentration in the blank from the concentration in the sample is not acceptable because the concentration of the background in the blank is highly variable.

## 12. DATA ANALYSIS AND CALCULATIONS

12.1 Complete chromatographic resolution is not necessary for accurate and precise measurements of analyte concentrations if unique ions with adequate intensities are available for quantitation.

12.1.1 Calculate analyte and surrogate concentrations.

$$C_x = \frac{(A_x)(Q_{is})\ 1000}{(A_{is})\ RF\ V}$$

where:

$C_x$ = concentration of analyte or surrogate in µg/L in the water sample.

$A_x$ = integrated abundance of the quantitation ion of the analyte in the sample.

$A_{is}$ = integrated abundance of the quantitation ion of the internal standard in the sample.

$Q_{is}$ = total quantity (in micrograms) of internal standard added to the water sample.

V = original water sample volume in mL.

RF = mean response factor of analyte from the initial calibration.

12.1.2 Alternatively, use the GC/MS system software or other available proven software to compute the concentrations of the analytes and surrogates from the linear or second order regression curves.

12.1.3 Calculations should utilize all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures (one digit of uncertainty). Experience indicates that three significant figures may be used for concentrations above 99 µg/L, two significant figures for concentrations between 1- 99 µg/L, and one significant figure for lower concentrations.

12.1.4 Calculate the total trihalomethane concentration by summing the four individual trihalomethane concentrations.

## 13. METHOD PERFORMANCE

13.1 Single laboratory accuracy and precision data were obtained for the method analytes using laboratory fortified blanks with analytes at concentrations between 1 and 5 µg/L. Results were obtained using the four columns specified (Sect. 6.3.2.1) and the open split or jet separator (Sect. 6.3.3.1), or the cryogenic interface (Sect. 6.3.3.2). These data are shown in Tables 4-8.

13.2 With these data, method detection limits were calculated using the formula (3):

$$MDL = S\ t_{(n-1,1-alpha = 0.99)}$$

where:

$t_{(n-1,1-alpha = 0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom,

n = number of replicates

S = the standard deviation of the replicate analyses.

## 14. POLLUTION PREVENTION

14.1 No solvents are utilized in this method except the extremely small volumes of methanol needed to make calibration standards. The only other chemicals used in this method are the neat materials in preparing standards and sample preservatives. All are used in extremely small amounts and pose no threat to the environment.

## 15. WASTE MANAGEMENT

15.1 There are no waste management issues involved with this method. Due to the nature of this method, the discarded samples are chemically less contaminated than when they were collected.

## 16. REFERENCES

1. A. Alford-Stevens, J.W. Eichelberger, W.L. Budde, "Purgeable Organic Compounds in Water by Gas Chromatography/Mass Spectrometry, Method 524." Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio, February 1983.

2. C. Madding, "Volatile Organic Compounds in Water by Purge and Trap Capillary Column GC/MS," Proceedings of the Water Quality Technology Conference, American Water Works Association, Denver, CO, December 1984.

3. J.A. Glaser, D.L. Foerst, G.D. McKee, S.A. Quave, and W.L. Budde, "Trace Analyses for Wastewaters, "Environ. Sci. Technol., 15, 1426, 1981.

4. "Carcinogens-Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.

5. "OSHA Safety and Health Standards, General Industry," (29CFR1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).

6. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

7. R.F. Arrendale, R.F. Severson, and O.T. Chortyk, "Open Split Interface for Capillary Gas Chromatography/Mass Spectrometry," Anal. Chem. 1984, 56, 1533.

8. J.J. Flesch, P.S. Fair, "The Analysis of Cyanogen Chloride in Drinking Water," Proceedings of Water Quality Technology Conference, American Water Works Association, St. Louis, MO., November 14-16, 1988.

## 17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

### TABLE 1. MOLECULAR WEIGHTS AND QUANTITATION IONS FOR METHOD ANALYTES

| Compound | MW[a] | Primary Quantitation Ion | Secondary Quantitation Ions |
|---|---|---|---|
| Internal standard | | | |
| Fluorobenzene | 96 | 96 | 77 |
| Surrogates | | | |
| 4-Bromofluorobenzene | 174 | 95 | 174,176 |
| 1,2-Dichlorobenzene-d4 | 150 | 152 | 115,150 |
| Target Analytes | | | |
| Acetone | 58 | 43 | 58 |
| Acrylonitrile | 53 | 52 | 53 |
| Allyl chloride | 76 | 76 | 49 |
| Benzene | 78 | 78 | 77 |
| Bromobenzene | 156 | 156 | 77,158 |
| Bromochloromethane | 128 | 128 | 49,130 |
| Bromodichloromethane | 162 | 83 | 85,127 |
| Bromoform | 250 | 173 | 175,252 |
| Bromomethane | 94 | 94 | 96 |
| 2-Butanone | 72 | 43 | 57,72 |
| n-Butylbenzene | 134 | 91 | 134 |
| sec-Butylbenzene | 134 | 105 | 134 |
| tert-Butylbenzene | 134 | 119 | 91 |
| Carbon disulfide | 76 | 76 | -- |
| Carbon tetrachloride | 152 | 117 | 119 |
| Chloroacetonitrile | 75 | 48 | 75 |
| Chlorobenzene | 112 | 112 | 77,114 |
| 1-Chlorobutane | 92 | 56 | 49 |
| Chloroethane | 64 | 64 | 66 |
| Chloroform | 118 | 83 | 85 |
| Chloromethane | 50 | 50 | 52 |
| 2-Chlorotoluene | 126 | 91 | 126 |
| 4-Chlorotoluene | 126 | 91 | 126 |
| Dibromochloromethane | 206 | 129 | 127 |
| 1,2-Dibromo-3-Chloropropane | 234 | 75 | 155,157 |
| 1,2-Dibromoethane | 186 | 107 | 109,188 |
| Dibromomethane | 172 | 93 | 95,174 |
| 1,2-Dichlorobenzene | 146 | 146 | 111,148 |
| 1,3-Dichlorobenzene | 146 | 146 | 111,148 |
| 1,4-Dichlorobenzene | 146 | 146 | 111,148 |

## TABLE 1. (continued)

| Compound | MW[a] | Primary Quantitation Ion | Secondary Quantitation Ions |
|---|---|---|---|
| trans-1,4-Dichloro-2-butene | 124 | 53 | 88,75 |
| Dichlorodifluoromethane | 120 | 85 | 87 |
| 1,1-Dichloroethane | 98 | 63 | 65,83 |
| 1,2-Dichloroethane | 98 | 62 | 98 |
| 1,1-Dichloroethene | 96 | 96 | 61,63 |
| cis-1,2-Dichloroethene | 96 | 96 | 61,98 |
| trans-1,2-Dichloroethene | 96 | 96 | 61,98 |
| 1,2-Dichloropropane | 112 | 63 | 112 |
| 1,3-Dichloropropane | 112 | 76 | 78 |
| 2,2-Dichloropropane | 112 | 77 | 97 |
| 1,1-Dichloropropene | 110 | 75 | 110,77 |
| 1,1-Dichloropropanone | 126 | 43 | 83 |
| cis-1,3-dichloropropene | 110 | 75 | 110 |
| trans-1,3-dichloropropene | 110 | 75 | 110 |
| Diethyl ether | 74 | 59 | 45,73 |
| Ethylbenzene | 106 | 91 | 106 |
| Ethyl methacrylate | 114 | 69 | 99 |
| Hexachlorobutadiene | 258 | 225 | 260 |
| Hexachloroethane | 234 | 117 | 119,201 |
| 2-Hexanone | 100 | 43 | 58 |
| Isopropylbenzene | 120 | 105 | 120 |
| 4-Isopropyltoluene | 134 | 119 | 134,91 |
| Methacrylonitrile | 67 | 67 | 52 |
| Methyl acrylate | 86 | 55 | 85 |
| Methylene chloride | 84 | 84 | 86,49 |
| Methyl iodide | 142 | 142 | 127 |
| Methylmethacrylate | 100 | 69 | 99 |
| 4-Methyl-2-pentanone | 100 | 43 | 58,85 |
| Methyl-t-butyl ether | 88 | 73 | 57 |
| Naphthalene | 128 | 128 | -- |
| Nitrobenzene | 123 | 51 | 77 |
| 2-Nitropropane | 89 | 46 | -- |
| Pentachloroethane | 200 | 117 | 119,167 |
| Propionitrile | 55 | 54 | -- |
| n-Propylbenzene | 120 | 91 | 120 |
| Styrene | 104 | 104 | 78 |
| 1,1,1,2-Tetrachloroethane | 166 | 131 | 133,119 |
| 1,1,2,2-Tetrachloroethane | 166 | 83 | 131,85 |
| Tetrachloroethene | 164 | 166 | 168,129 |
| Tetrahydrofuran | 72 | 71 | 72,42 |
| Toluene | 92 | 92 | 91 |
| 1,2,3-Trichlorobenzene | 180 | 180 | 182 |
| 1,2,4-Trichlorobenzene | 180 | 180 | 182 |
| 1,1,1-Trichloroethane | 132 | 97 | 99,61 |
| 1,1,2-Trichloroethane | 132 | 83 | 97,85 |

TABLE 1. (continued)

| Compound | MW[a] | Primary Quantitation Ion | Secondary Quantitation Ions |
|---|---|---|---|
| Trichloroethene | 130 | 95 | 130,132 |
| Trichlorofluoromethane | 136 | 101 | 103 |
| 1,2,3-Trichloropropane | 146 | 75 | 77 |
| 1,2,4-Trimethylbenzene | 120 | 105 | 120 |
| 1,3,5-Trimethylbenzene | 120 | 105 | 120 |
| Vinyl Chloride | 62 | 62 | 64 |
| o-Xylene | 106 | 106 | 91 |
| m-Xylene | 106 | 106 | 91 |
| p-Xylene | 106 | 106 | 91 |

[a] Monoisotopic molecular weight calculated from the atomic masses of the isotopes with the smallest masses.

## TABLE 2. CHROMATOGRAPHIC RETENTION TIMES FOR METHOD ANALYTES ON THREE COLUMNS WITH FOUR SETS OF CONDITIONS[a]

| | Retention Time (min:sec) | | | | |
|---|---|---|---|---|---|
| Compound | Column 1[b] | Column 2[b] | Column 2[c] | Column 3[d] | Column 4[e] |
| Internal standard | | | | | |
| Fluorobenzene | 8:49 | 6:27 | 14:06 | 8:03 | 22:00 |
| Surrogates | | | | | |
| 4-Bromofluorobenzene | 18:38 | 15:43 | 23:38 | | 31:21 |
| 1,2-Dichlorobenzene-d4 | 22:16 | 19:08 | 27:25 | | 35:51 |
| Target Analytes | | | | | |
| Acetone | | | | | 16:14 |
| Acrylonitrile | | | | | 17:49 |
| Allyl chloride | | | | | 16:58 |
| Benzene | 8:14 | 5:40 | 13:30 | 7:25 | 21:32 |
| Bromobenzene | 18:57 | 15:52 | 24:00 | 16:25 | 31:52 |
| Bromochloromethane | 6:44 | 4:23 | 12:22 | 5:38 | 20:20 |
| Bromodichloromethane | 10:35 | 8:29 | 15:48 | 9:20 | 23:36 |
| Bromoform | 17:56 | 14:53 | 22:46 | 15:42 | 30:32 |
| Bromomethane | 2:01 | 0:58 | 4:48 | 1:17 | 12:26 |
| 2-Butanone | | | | | 19:41 |
| n-Butylbenzene | 22:13 | 19:29 | 27:32 | 17:57 | 35:41 |
| sec-Butylbenzene | 20:47 | 18:05 | 26:08 | 17:28 | 34:04 |
| tert-Butylbenzene | 20:17 | 17:34 | 25:36 | 17:19 | 33:26 |
| Carbon Disulfide | | | | | 16:30 |
| Carbon Tetrachloride | 7:37 | 5:16 | 13:10 | 7:25 | 21:11 |
| Chloroacetonitrile | | | | | 23:51 |
| Chlorobenzene | 15:46 | 13:01 | 20:40 | 14:20 | 28:26 |
| 1-Chlorobutane | | | | | 21:00 |
| Chloroethane | 2:05 | 1:01 | | 1:27 | |
| Chloroform | 6:24 | 4:48 | 12:36 | 5:33 | 20:27 |
| Chloromethane | 1:38 | 0:44 | 3:24 | 0:58 | 9:11 |
| 2-Chlorotoluene | 19:20 | 16:25 | 24:32 | 16:44 | 32:21 |
| 4-Chlorotoluene | 19:30 | 16:43 | 24:46 | 16:49 | 32:38 |
| Cyanogen chloride (8) | | | | 1:03 | |
| Dibromochloromethane | 14:23 | 11:51 | 19:12 | 12:48 | 26:57 |
| 1,2-Dibromo-3-Chloropropane | 24:32 | 21:05 | | 18:02 | 38:20 |
| 1,2-Dibromoethane | 14:44 | 11:50 | 19:24 | 13:36 | 27:19 |
| Dibromomethane | 10:39 | 7:56 | 15:26 | 9:05 | 23:22 |
| 1,2-Dichlorobenzene | 22:31 | 19:10 | 27:26 | 17:47 | 35:55 |
| 1,3-Dichlorobenzene | 21:13 | 18:08 | 26:22 | 17:28 | 34:31 |
| 1,4-Dichlorobenzene | 21:33 | 18:23 | 26:36 | 17:38 | 34:45 |
| t-1,4-Dichloro-2-butene | | | | | 31:44 |
| Dichlorodifluoromethane | 1:33 | 0:42 | 3:08 | 0:53 | 7:16 |
| 1,1-Dichloroethane | 4:51 | 2:56 | 10:48 | 4:02 | 18:46 |

## TABLE 2. (continued)

| Compound | Retention Time (min:sec) | | | | |
|---|---|---|---|---|---|
| | Column 1$^b$ | Column 2$^b$ | Column 2$^c$ | Column 3$^d$ | Column 4$^e$ |
| 1,2-Dichloroethane | 8:24 | 5:50 | 13:38 | 7:00 | 21:31 |
| 1,1-Dichloroethene | 2:53 | 1:34 | 7:50 | 2:20 | 16:01 |
| cis-1,2-Dichloroethene | 6:11 | 3:54 | 11:56 | 5:04 | 19:53 |
| trans-1,2-Dichloroethene | 3:59 | 2:22 | 9:54 | 3:32 | 17:54 |
| 1,2-Dichloropropane | 10:05 | 7:40 | 15:12 | 8:56 | 23:08 |
| 1,3-Dichloropropane | 14:02 | 11:19 | 18:42 | 12:29 | 26:23 |
| 2,2-Dichloropropane | 6:01 | 3:48 | 11:52 | 5:19 | 19:54 |
| 1,1-Dichloropropanone | | | | | 24:52 |
| 1,1-Dichloropropene | 7:49 | 5:17 | 13:06 | 7:10 | 21:08 |
| cis-1,3-dichloropropene | 11.58 | | 16:42 | | 24:24 |
| trans-1,3-dichloropropene | 13.46 | | 17:54 | | 25:33 |
| Diethyl ether | | | | | 15:31 |
| Ethylbenzene | 15:59 | 13:23 | 21:00 | 14:44 | 28:37 |
| Ethyl Methacrylate | | | | | 25:35 |
| Hexachlorobutadiene | 26:59 | 23:41 | 32:04 | 19:14 | 42:03 |
| Hexachloroethane | | | | | 36:45 |
| Hexanone | | | | | 26:23 |
| Isopropylbenzene | 18:04 | 15:28 | 23:18 | 16:25 | 30:52 |
| 4-Isopropyltoluene | 21:12 | 18:31 | 26:30 | 17:38 | 34:27 |
| Methacrylonitrile | | | | | 20:15 |
| Methylacrylate | | | | | 20:02 |
| Methylene Chloride | 3:36 | 2:04 | 9:16 | 2:40 | 17:18 |
| Methyl Iodide | | | | | 16:21 |
| Methylmethacrylate | | | | | 23:08 |
| 4-Methyl-2-pentanone | | | | | 24:38 |
| Methyl-t-butyl ether | | | | | 17:56 |
| Naphthalene | 27:10 | 23:31 | 32:12 | 19:04 | 42:29 |
| Nitrobenzene | | | | | 39:02 |
| 2-Nitropropane | | | | | 23:58 |
| Pentachloroethane | | | | | 33:33 |
| Propionitrile | | | | | 19:58 |
| n-Propylbenzene | 19:04 | 16:25 | 24:20 | 16:49 | 32:00 |
| Styrene | 17:19 | 14:36 | 22:24 | 15:47 | 29:57 |
| 1,1,1,2-Tetrachloroethane | 15:56 | 13:20 | 20:52 | 14:44 | 28:35 |
| 1,1,2,2-Tetrachloroethane | 18:43 | 16:21 | 24:04 | 15:47 | 31:35 |
| Tetrachloroethene | 13:44 | 11:09 | 18:36 | 13:12 | 26:27 |
| Tetrahydrofuran | | | | | 20:26 |
| Toluene | 12:26 | 10:00 | 17:24 | 11:31 | 25:13 |
| 1,2,3-Trichlorobenzene | 27:47 | 24:11 | 32:58 | 19:14 | 43:31 |
| 1,2,4-Trichlorobenzene | 26:33 | 23:05 | 31:30 | 18:50 | 41:26 |
| 1,1,1-Trichloroethane | 7:16 | 4:50 | 12:50 | 6:46 | 20:51 |
| 1,1,2-Trichloroethane | 13:25 | 11:03 | 18:18 | 11:59 | 25:59 |
| Trichloroethene | 9:35 | 7:16 | 14:48 | 9:01 | 22:42 |
| Trichlorofluoromethane | 2:16 | 1:11 | 6:12 | 1:46 | 14:18 |
| 1,2,3-Trichloropropane | 19:01 | 16:14 | 24:08 | 16:16 | 31:47 |
| 1,2,4-Trimethylbenzene | 20:20 | 17:42 | 31:30 | 17:19 | 33:33 |

TABLE 2. (continued)

| Compound | Retention Column 1[b] | Time Column 2[b] | (min:sec) Column 2[c] | Column 3[d] | Column 4[e] |
|---|---|---|---|---|---|
| 1,3,5-Trimethylbenzene | 19:28 | 16:54 | 24:50 | 16:59 | 32:26 |
| Vinyl chloride | 1:43 | 0:47 | 3:56 | 1:02 | 10:22 |
| o-Xylene | 17:07 | 14:31 | 22:16 | 15:47 | 29:56 |
| m-Xylene | 16:10 | 13:41 | 21:22 | 15:18 | 28:53 |
| p-Xylene | 16:07 | 13:41 | 21:18 | 15:18 | 28:53 |

[a] Columns 1-4 are those given in Sect. 6.3.2.1; retention times were measured from the beginning of thermal desorption from the trap (columns 1-2, and 4) or from the beginning of thermal release from the cryogenic interface (column 3).

[b] GC conditions given in Sect. 11.3.1.

[c] GC conditions given in Sect. 11.3.2.

[d] GC conditions given in Sect. 11.3.3.

[e] GC conditions given in Sect. 11.3.4.

TABLE 3. ION ABUNDANCE CRITERIA FOR 4-BROMOFLUOROBENZENE (BFB)

| Mass (M/z) | Relative Abundance Criteria |
|---|---|
| 50 | 15 to 40% of mass 95 |
| 75 | 30 to 80% of mass 95 |
| 95 | Base Peak, 100% Relative Abundance |
| 96 | 5 to 9% of mass 95 |
| 173 | < 2% of mass 174 |
| 174 | > 50% of mass 95 |
| 175 | 5 to 9% of mass 174 |
| 176 | > 95% but < 101% of mass 174 |
| 177 | 5 to 9% of mass 176 |

TABLE 4. ACCURACY AND PRECISION DATA FROM 16-31 DETERMINATIONS OF THE METHOD ANALYTES IN REAGENT WATER USING WIDE BORE CAPILLARY COLUMN 1[a]

| Compound | True Conc. Range (µg/L) | Mean Accuracy (% of True Value) | Rel. Std. Dev. (%) | Method Det. Limit[b] (µg/L) |
|---|---|---|---|---|
| Benzene | 0.1-10 | 97 | 5.7 | 0.04 |
| Bromobenzene | 0.1-10 | 100 | 5.5 | 0.03 |
| Bromochloromethane | 0.5-10 | 90 | 6.4 | 0.04 |
| Bromodichloromethane | 0.1-10 | 95 | 6.1 | 0.08 |
| Bromoform | 0.5-10 | 101 | 6.3 | 0.12 |
| Bromomethane | 0.5-10 | 95 | 8.2 | 0.11 |
| n-Butylbenzene | 0.5-10 | 100 | 7.6 | 0.11 |
| sec-Butylbenzene | 0.5-10 | 100 | 7.6 | 0.13 |
| tert-Butylbenzene | 0.5-10 | 102 | 7.3 | 0.14 |
| Carbon tetrachloride | 0.5-10 | 84 | 8.8 | 0.21 |
| Chlorobenzene | 0.1-10 | 98 | 5.9 | 0.04 |
| Chloroethane | 0.5-10 | 89 | 9.0 | 0.10 |
| Chloroform | 0.5-10 | 90 | 6.1 | 0.03 |
| Chloromethane | 0.5-10 | 93 | 8.9 | 0.13 |
| 2-Chlorotoluene | 0.1-10 | 90 | 6.2 | 0.04 |
| 4-Chlorotoluene | 0.1-10 | 99 | 8.3 | 0.06 |
| Dibromochloromethane | 0.1-10 | 92 | 7.0 | 0.05 |
| 1,2-Dibromo-3-chloropropane | 0.5-10 | 83 | 19.9 | 0.26 |
| 1,2-Dibromoethane | 0.5-10 | 102 | 3.9 | 0.06 |
| Dibromomethane | 0.5-10 | 100 | 5.6 | 0.24 |
| 1,2-Dichlorobenzene | 0.1-10 | 93 | 6.2 | 0.03 |
| 1,3-Dichlorobenzene | 0.5-10 | 99 | 6.9 | 0.12 |
| 1,4-Dichlorobenzene | 0.2-20 | 103 | 6.4 | 0.03 |
| Dichlorodifluoromethane | 0.5-10 | 90 | 7.7 | 0.10 |
| 1,1-Dichloroethane | 0.5-10 | 96 | 5.3 | 0.04 |
| 1,2-Dichloroethane | 0.1-10 | 95 | 5.4 | 0.06 |
| 1,1-Dichloroethene | 0.1-10 | 94 | 6.7 | 0.12 |
| cis-1,2 Dichloroethene | 0.5-10 | 101 | 6.7 | 0.12 |
| trans-1,2-Dichloroethene | 0.1-10 | 93 | 5.6 | 0.06 |
| 1,2-Dichloropropane | 0.1-10 | 97 | 6.1 | 0.04 |
| 1,3-Dichloropropane | 0.1-10 | 96 | 6.0 | 0.04 |
| 2,2-Dichloropropane | 0.5-10 | 86 | 16.9 | 0.35 |
| 1,1-Dichloropropene | 0.5-10 | 98 | 8.9 | 0.10 |
| cis-1,2-Dichloropropene | | | | |
| trans-1,2-Dichloropropene | | | | |
| Ethylbenzene | 0.1-10 | 99 | 8.6 | 0.06 |
| Hexachlorobutadiene | 0.5-10 | 100 | 6.8 | 0.11 |
| Isopropylbenzene | 0.5-10 | 101 | 7.6 | 0.15 |
| 4-Isopropyltoluene | 0.1-10 | 99 | 6.7 | 0.12 |
| Methylene chloride | 0.1-10 | 95 | 5.3 | 0.03 |
| Naphthalene | 0.1-100 | 104 | 8.2 | 0.04 |
| n-Propylbenzene | 0.1-10 | 100 | 5.8 | 0.04 |
| Styrene | 0.1-100 | 102 | 7.2 | 0.04 |

## TABLE 4. (Continued)

| Compound | True Conc. Range (μg/L) | Mean Accuracy (% of True Value) | Rel. Std. Dev. (%) | Method Det. Limit[b] (μg/L) |
|---|---|---|---|---|
| 1,1,1,2-Tetrachloroethane | 0.5-10 | 90 | 6.8 | 0.05 |
| 1,1,2,2-Tetrachloroethane | 0.1-10 | 91 | 6.3 | 0.04 |
| Tetrachloroethene | 0.5-10 | 89 | 6.8 | 0.14 |
| Toluene | 0.5-10 | 102 | 8.0 | 0.11 |
| 1,2,3-Trichlorobenzene | 0.5-10 | 109 | 8.6 | 0.03 |
| 1,2,4-Trichlorobenzene | 0.5-10 | 108 | 8.3 | 0.04 |
| 1,1,1-Trichloroethane | 0.5-10 | 98 | 8.1 | 0.08 |
| 1,1,2-Trichloroethane | 0.5-10 | 104 | 7.3 | 0.10 |
| Trichloroethene | 0.5-10 | 90 | 7.3 | 0.19 |
| Trichlorofluoromethane | 0.5-10 | 89 | 8.1 | 0.08 |
| 1,2,3-Trichloropropane | 0.5-10 | 108 | 14.4 | 0.32 |
| 1,2,4-Trimethylbenzene | 0.5-10 | 99 | 8.1 | 0.13 |
| 1,3,5-Trimethylbenzene | 0.5-10 | 92 | 7.4 | 0.05 |
| Vinyl chloride | 0.5-10 | 98 | 6.7 | 0.17 |
| o-Xylene | 0.1-31 | 103 | 7.2 | 0.11 |
| m-Xylene | 0.1-10 | 97 | 6.5 | 0.05 |
| p-Xylene | 0.5-10 | 104 | 7.7 | 0.13 |

[a] Data obtained by using column 1 with a jet separator interface and a quadrupole mass spectrometer (Sect. 11.3.1) with analytes divided among three solutions.

[b] Replicate samples at the lowest concentration listed in column 2 of this table were analyzed. These results were used to calculate MDLs.

## TABLE 5. ACCURACY AND PRECISION DATA FROM SEVEN DETERMINATIONS OF METHOD ANALYTES IN REAGENT WATER USING THE CRYOGENIC TRAPPING OPTION AND A NARROW BORE CAPILLARY COLUMN 3[a]

| Compound | True Conc. (µg/L) | Mean Accuracy (% of True Value) | Rel. Std. Dev. (%) | Method Dect. Limit (µg/L) |
|---|---|---|---|---|
| Benzene | 0.1 | 99 | 6.2 | 0.03 |
| Bromobenzene | 0.5 | 97 | 7.4 | 0.11 |
| Bromochloromethane | 0.5 | 97 | 5.8 | 0.07 |
| Bromodichloromethane | 0.1 | 100 | 4.6 | 0.03 |
| Bromoform | 0.1 | 99 | 5.4 | 0.20 |
| Bromomethane | 0.1 | 99 | 7.1 | 0.06 |
| n-Butylbenzene | 0.5 | 94 | 6.0 | 0.03 |
| sec-Butylbenzene | 0.5 | 90 | 7.1 | 0.12 |
| tert-Butylbenzene | 0.5 | 90 | 2.5 | 0.33 |
| Carbon tetrachloride | 0.1 | 92 | 6.8 | 0.08 |
| Chlorobenzene | 0.1 | 91 | 5.8 | 0.03 |
| Chloroethane | 0.1 | 100 | 5.8 | 0.02 |
| Chloroform | 0.1 | 95 | 3.2 | 0.02 |
| Chloromethane | 0.1 | 99 | 4.7 | 0.05 |
| 2-Chlorotoluene | 0.1 | 99 | 4.6 | 0.05 |
| 4-Chlorotoluene | 0.1 | 96 | 7.0 | 0.05 |
| Cyanogen chloride[b] |  | 92 | 10.6 | 0.30 |
| Dibromochloromethane | 0.1 | 99 | 5.6 | 0.07 |
| 1,2-Dibromo-3-chloropropane | 0.1 | 92 | 10.0 | 0.05 |
| 1,2-Dibromoethane | 0.1 | 97 | 5.6 | 0.02 |
| Dibromomethane | 0.1 | 93 | 6.9 | 0.03 |
| 1,2-Dichlorobenzene | 0.1 | 97 | 3.5 | 0.05 |
| 1,3-Dichlorobenzene | 0.1 | 99 | 6.0 | 0.05 |
| 1,4-Dichlorobenzene | 0.1 | 93 | 5.7 | 0.04 |
| Dichlorodifluoromethane | 0.1 | 99 | 8.8 | 0.11 |
| 1,1-Dichloroethane | 0.1 | 98 | 6.2 | 0.03 |
| 1,2-Dichloroethane | 0.1 | 100 | 6.3 | 0.02 |
| 1,1-Dichloroethene | 0.1 | 95 | 9.0 | 0.05 |
| cis-1,2 Dichloroethene | 0.1 | 100 | 3.7 | 0.06 |
| trans-1,2-Dichloroethene | 0.1 | 98 | 7.2 | 0.03 |
| 1,2-Dichloropropane | 0.1 | 96 | 6.0 | 0.02 |
| 1,3-Dichloropropane | 0.1 | 99 | 5.8 | 0.04 |
| 2,2-Dichloropropane | 0.1 | 99 | 4.9 | 0.05 |
| 1,1-Dichloropropene | 0.1 | 98 | 7.4 | 0.02 |
| cis-1,3-Dichloropropene |  |  |  |  |
| trans-1,3-Dichloropropene |  |  |  |  |
| Ethylbenzene | 0.1 | 99 | 5.2 | 0.03 |
| Hexachlorobutadiene | 0.1 | 100 | 6.7 | 0.04 |
| Isopropylbenzene | 0.5 | 98 | 6.4 | 0.10 |
| 4-Isopropyltoluene | 0.5 | 87 | 13.0 | 0.26 |
| Methylene chloride | 0.5 | 97 | 13.0 | 0.09 |
| Naphthalene | 0.1 | 98 | 7.2 | 0.04 |

## TABLE 5. (Continued)

| Compound | True Conc. (µg/L) | Mean Accuracy (% of True Value) | Rel. Std. Dev. (%) | Method Dect. Limit (µg/L) |
|---|---|---|---|---|
| n-Propylbenzene | 0.1 | 99 | 6.6 | 0.06 |
| Styrene | 0.1 | 96 | 19.0 | 0.06 |
| 1,1,1,2-Tetrachloroethane | 0.1 | 100 | 4.7 | 0.04 |
| 1,1,2,2-Tetrachloroethane | 0.5 | 100 | 12.0 | 0.20 |
| Tetrachloroethene | 0.1 | 96 | 5.0 | 0.05 |
| Toluene | 0.1 | 100 | 5.9 | 0.08 |
| 1,2,3-Trichlorobenzene | 0.1 | 98 | 8.9 | 0.04 |
| 1,2,4-Trichlorobenzene | 0.1 | 91 | 16.0 | 0.20 |
| 1,1,1-Trichloroethane | 0.1 | 100 | 4.0 | 0.04 |
| 1,1,2-Trichloroethane | 0.1 | 98 | 4.9 | 0.03 |
| Trichloroethene | 0.1 | 96 | 2.0 | 0.02 |
| Trichlorofluoromethane | 0.1 | 97 | 4.6 | 0.07 |
| 1,2,3-Trichloropropane | 0.1 | 96 | 6.5 | 0.03 |
| 1,2,4-Trimethylbenzene | 0.1 | 96 | 6.5 | 0.04 |
| 1,3,5-Trimethylbenzene | 0.1 | 99 | 4.2 | 0.02 |
| Vinyl chloride | 0.1 | 96 | 0.2 | 0.04 |
| o-Xylene | 0.1 | 94 | 7.5 | 0.06 |
| m-Xylene | 0.1 | 94 | 4.6 | 0.03 |
| p-Xylene | 0.1 | 97 | 6.1 | 0.06 |

---

[a] Data obtained by using column 3 with a cryogenic interface and a quadrupole mass spectrometer (Sect 11.3.3).

[b] Reference 8.

## TABLE 6. ACCURACY AND PRECISION DATA FROM SEVEN DETERMINATIONS OF THE METHOD ANALYTES IN REAGENT WATER USING WIDE BORE CAPILLARY COLUMN 2[a]

| Compound | No.[b] | Mean Accuracy (% of True Value, 2 µg/L Conc.) | RSD (%) | Mean Accuracy (% of True Value, 0.2 µg/L Conc.) | RSD (%) |
|---|---|---|---|---|---|
| Internal Standard | | | | | |
| Fluorobenzene | 1 | - | - | - | - |
| Surrogates | | | | | |
| 4-Bromofluorobenzene | 2 | 98 | 1.8 | 96 | 1.3 |
| 1,2-Dichlorobenzene-$d_4$ | 3 | 97 | 3.2 | 95 | 1.7 |
| Target Analytes | | | | | |
| Benzene | 37 | 97 | 4.4 | 113 | 1.8 |
| Bromobenzene | 38 | 102 | 3.0 | 101 | 1.9 |
| Bromochloromethane | 4 | 99 | 5.2 | 102 | 2.9 |
| Bromodichloromethane | 5 | 96 | 1.8 | 100 | 1.8 |
| Bromoform | 6 | 89 | 2.4 | 90 | 2.2 |
| Bromomethane | 7 | 55 | 27. | 52 | 6.7 |
| n-Butylbenzene | 39 | 89 | 4.8 | 87 | 2.3 |
| sec-Butylbenzene | 40 | 102 | 3.5 | 100 | 2.8 |
| tert-Butylbenzene | 41 | 101 | 4.5 | 100 | 2.9 |
| Carbon tetrachloride | 8 | 84 | 3.2 | 92 | 2.6 |
| Chlorobenzene | 42 | 104 | 3.1 | 103 | 1.6 |
| Chloroethane[c] | | | | | |
| Chloroform | 9 | 97 | 2.0 | 95 | 2.1 |
| Chloromethane | 10 | 110 | 5.0 | d | |
| 2-Chlorotoluene | 43 | 91 | 2.4 | 108 | 3.1 |
| 4-Chlorotoluene | 44 | 89 | 2.0 | 108 | 4.4 |
| Dibromochloromethane | 11 | 95 | 2.7 | 100 | 3.0 |
| 1,2-Dibromo-3-chloropropane[c] | | | | | |
| 1,2-Dibromoethane[c] | | | | | |
| Dibromomethane | 13 | 99 | 2.1 | 95 | 2.2 |
| 1,2-Dichlorobenzene | 45 | 93 | 2.7 | 94 | 5.1 |
| 1,3-Dichlorobenzene | 46 | 100 | 4.0 | 87 | 2.3 |
| 1,4-Dichlorobenzene | 47 | 98 | 4.1 | 94 | 2.8 |
| Dichlorodifluoromethane | 14 | 38 | 25. | d | |
| 1,1-Dichloroethane | 15 | 97 | 2.3 | 85 | 3.6 |
| 1,2-Dichloroethane | 16 | 102 | 3.8 | 100 | 2.1 |
| 1,1-Dichloroethene | 17 | 90 | 2.2 | 87 | 3.8 |
| cis-1,2-Dichloroethene | 18 | 100 | 3.4 | 89 | 2.9 |
| trans-1,2-Dichloroethene | 19 | 92 | 2.1 | 85 | 2.3 |

## TABLE 6. (Continued)

| Compound | No.[b] | Mean Accuracy (% of True Value, 2 µg/L Conc.) | RSD (%) | Mean Accuracy (% of True Value, 0.2 µg/L Conc.) | RSD (%) |
|---|---|---|---|---|---|
| 1,2-Dichloropropane | 20 | 102 | 2.2 | 103 | 2.9 |
| 1,3-Dichloropropane | 21 | 92 | 3.7 | 93 | 3.2 |
| 2,2-Dichloropropane[c] | | | | | |
| 1,1-Dichloropropene[c] | | | | | |
| cis-1,3-Dichloropropene[c] | | | | | |
| trans-1,3-Dichloropropene | 25 | 96 | 1.7 | 99 | 2.1 |
| Ethylbenzene | 48 | 96 | 9.1 | 100 | 4.0 |
| Hexachlorobutadiene | 26 | 91 | 5.3 | 88 | 2.4 |
| Isopropylbenzene | 49 | 103 | 3.2 | 101 | 2.1 |
| 4-Isopropyltoluene | 50 | 95 | 3.6 | 95 | 3.1 |
| Methylene chloride | 27 | e | | e | |
| Naphthalene | 51 | 93 | 7.6 | 78 | 8.3 |
| n-Propylbenzene | 52 | 102 | 4.9 | 97 | 2.1 |
| Styrene | 53 | 95 | 4.4 | 104 | 3.1 |
| 1,1,1,2-Tetrachloroethane | 28 | 99 | 2.7 | 95 | 3.8 |
| 1,1,2,2-Tetrachloroethane | 29 | 101 | 4.6 | 84 | 3.6 |
| Tetrachloroethene | 30 | 97 | 4.5 | 92 | 3.3 |
| Toluene | 54 | 105 | 2.8 | 126 | 1.7 |
| 1,2,3-Trichlorobenzene | 55 | 90 | 5.7 | 78 | 2.9 |
| 1,2,4-Trichlorobenzene | 56 | 92 | 5.2 | 83 | 5.9 |
| 1,1,1-Trichloroethane | 31 | 94 | 3.9 | 94 | 2.5 |
| 1,1,2-Trichloroethane | 32 | 107 | 3.4 | 109 | 2.8 |
| Trichloroethene | 33 | 99 | 2.9 | 106 | 2.5 |
| Trichlorofluoromethane | 34 | 81 | 4.6 | 48 | 13. |
| 1,2,3-Trichloropropane | 35 | 97 | 3.9 | 91 | 2.8 |
| 1,2,4-Trimethylbenzene | 57 | 93 | 3.1 | 106 | 2.2 |
| 1,3,5-Trimethylbenzene | 58 | 88 | 2.4 | 97 | 3.2 |
| Vinyl chloride | 36 | 104 | 3.5 | 115 | 14. |
| o-Xylene | 59 | 97 | 1.8 | 98 | 1.7 |
| m-Xylene | 60 | f | | f | |
| p-Xylene | 61 | 98 | 2.3 | 103 | 1.4 |

---

[a] Data obtained using column 2 with the open split interface and an ion trap mass spectrometer (Sect. 11.3.2) with all method analytes in the same reagent water solution.

[b] Designation in Figures 1 and 2.

[c] Not measured; authentic standards were not available.

[d] Not found at 0.2 µg/L.

[e] Not measured; methylene chloride was in the laboratory reagent blank.

[f] m-xylene coelutes with and cannot be distinguished from its isomer p-xylene, No 61.

## TABLE 7. ACCURACY AND PRECISION DATA FROM SEVEN DETERMINATIONS OF METHOD ANALYTES IN REAGENT WATER USING WIDE BORE CAPILLARY COLUMN 4

| Compound | True Conc. (µg/L) | Mean Conc. Detected (ug/L) | Rel. Std. Dev. (%) | Method Det. Limit (µg/L) |
|---|---|---|---|---|
| Acetone | 1.0 | 1.6 | 5.7% | 0.28 |
| Acrylonitrile | 1.0 | 0.81 | 8.7% | 0.22 |
| Allyl chloride | 1.0 | 0.90 | 4.7% | 0.13 |
| 2-Butanone | 2.0 | 2.7 | 5.6% | 0.48 |
| Carbon disulfide | 0.20 | 0.19 | 15% | 0.093 |
| Chloroacetonitrile | 1.0 | 0.83 | 4.7% | 0.12 |
| 1-Chlorobutane | 1.0 | 0.87 | 6.6% | 0.18 |
| t-1,2-Dichloro-2-butene | 1.0 | 1.3 | 8.7% | 0.36 |
| 1,1-Dichloropropanone | 5.0 | 4.2 | 7.7% | 1.0 |
| Diethyl ether | 1.0 | 0.92 | 9.5% | 0.28 |
| Ethyl methacrylate | 0.20 | 0.23 | 3.9% | 0.028 |
| Hexachloroethane | 0.20 | 0.18 | 10% | 0.057 |
| 2-Hexanone | 1.0 | 1.1 | 12% | 0.39 |
| Methacrylonitrile | 1.0 | 0.92 | 4.2% | 0.12 |
| Methylacrylate | 1.0 | 1.2 | 12% | 0.45 |
| Methyl iodide | 0.20 | 0.19 | 3.1% | 0.019 |
| Methylmethacrylate | 1.0 | 1.0 | 13% | 0.43 |
| 4-Methyl-2-pentanone | 0.40 | 0.56 | 9.7% | 0.17 |
| Methyl-tert-butylether | 0.40 | 0.52 | 5.6% | 0.090 |
| Nitrobenzene | 2.0 | 2.1 | 18% | 1.2 |
| 2-Nitropropane | 1.0 | 0.83 | 6.2% | 0.16 |
| Pentachloroethane | 0.20 | 0.23 | 20% | 0.14 |
| Propionitrile | 1.0 | 0.87 | 5.3% | 0.14 |
| Tetrahydrofuran | 5.0 | 3.9 | 13% | 1.6 |

TABLE 8. ACCURACY AND PRECISION FROM FOUR DETERMINATIONS OF METHOD ANALYTES IN THREE WATER MATRICES FORTIFIED AT 20 µG/L

| | REAGENT WATER | | | RAW WATER | | | TAP WATER | | |
|---|---|---|---|---|---|---|---|---|---|
| Compound | Mean (µg/L) | Dev. (%) | (% of True Value) | Mean (µg/L) | Dev. (%) | (% of True Value) | Mean (µg/L) | Dev. (%) | (% of True Value) |
| Acetone | 19 | 12% | 95% | 21 | 3.7% | 105% | 22 | 8.2% | 110% |
| Acrylonitrile | 20 | 4.7% | 100% | 22 | 3.4% | 110% | 21 | 1.3% | 105% |
| Allyl chloride | 20 | 5.1% | 100% | 20 | 2.8% | 100% | 19 | 3.5% | 95% |
| 2-Butanone | 17 | 11% | 85% | 19 | 7.3% | 95% | 17 | 5.6% | 85% |
| Carbon disulfide | 19 | 6.4% | 95% | 18 | 2.5% | 90% | 18 | 3.0% | 90% |
| Chloroacetonitrile | 20 | 4.1% | 100% | 23 | 4.7% | 115% | 23 | 1.3% | 115% |
| 1-Chlorobutane | 18 | 6.4% | 90% | 19 | 2.2% | 95% | 17 | 2.2% | 85% |
| t-1,2-Dichloro-2-butene | 19 | 4.1% | 95% | 22 | 2.9% | 110% | 21 | 0.90% | 105% |
| 1,1-Dichloropropanone | 20 | 5.6% | 100% | 22 | 6.4% | 110% | 21 | 7.7% | 105% |
| Diethyl ether | 18 | 6.7% | 90% | 22 | 3.4% | 110% | 22 | 2.6% | 110% |
| Ethyl methacrylate | 20 | 3.7% | 100% | 23 | 2.6% | 115% | 22 | 1.8% | 110% |
| Hexachloroethane | 20 | 6.1% | 100% | 21 | 2.5% | 105% | 21 | 2.0% | 105% |
| 2-Hexanone | 19 | 6.3% | 95% | 21 | 3.8% | 105% | 21 | 4.0% | 105% |
| Methacrylonitrile | 20 | 3.4% | 100% | 23 | 2.9% | 115% | 22 | 2.0% | 110% |
| Methylacrylate | 20 | 3.7% | 100% | 22 | 3.1% | 110% | 21 | 2.1% | 105% |

## TABLE 8 (Continued)

| Compound | REAGENT WATER Mean (µg/L) | Dev. (%) | (% of True Value) | RAW WATER Mean (µg/L) | Dev. (%) | (% of True Value) | TAP WATER Mean (µg/L) | Dev. (%) | (% of True Value) |
|---|---|---|---|---|---|---|---|---|---|
| Methyl iodide | 20 | 4.4% | 100% | 19 | 3.8% | 95% | 19 | 3.0% | 95% |
| Methylmethacrylate | 20 | 3.7% | 100% | 23 | 3.3% | 115% | 23 | 2.7% | 115% |
| 4-Methyl-2-pentanone | 19 | 8.7% | 95% | 21 | 5.5% | 105% | 22 | 7.2% | 110% |
| Methyl-tert-butylether | 19 | 3.5% | 95% | 22 | 2.5% | 110% | 22 | 3.6% | 110% |
| Nitrobenzene | 20 | 5.4% | 100% | 22 | 4.8% | 110% | 21 | 2.4% | 105% |
| 2-Nitropropane | 20 | 6.1% | 100% | 23 | 5.1% | 115% | 22 | 3.2% | 110% |
| Pentachloroethane | 19 | 5.2% | 95% | 21 | 2.6% | 105% | 22 | 1.7% | 110% |
| Propionitrile | 20 | 4.5% | 100% | 23 | 3.9% | 115% | 23 | 2.4% | 115% |
| Tetrahydrofuran | 20 | 2.8% | 100% | 24 | 3.2% | 120% | 21 | 2.9% | 105% |

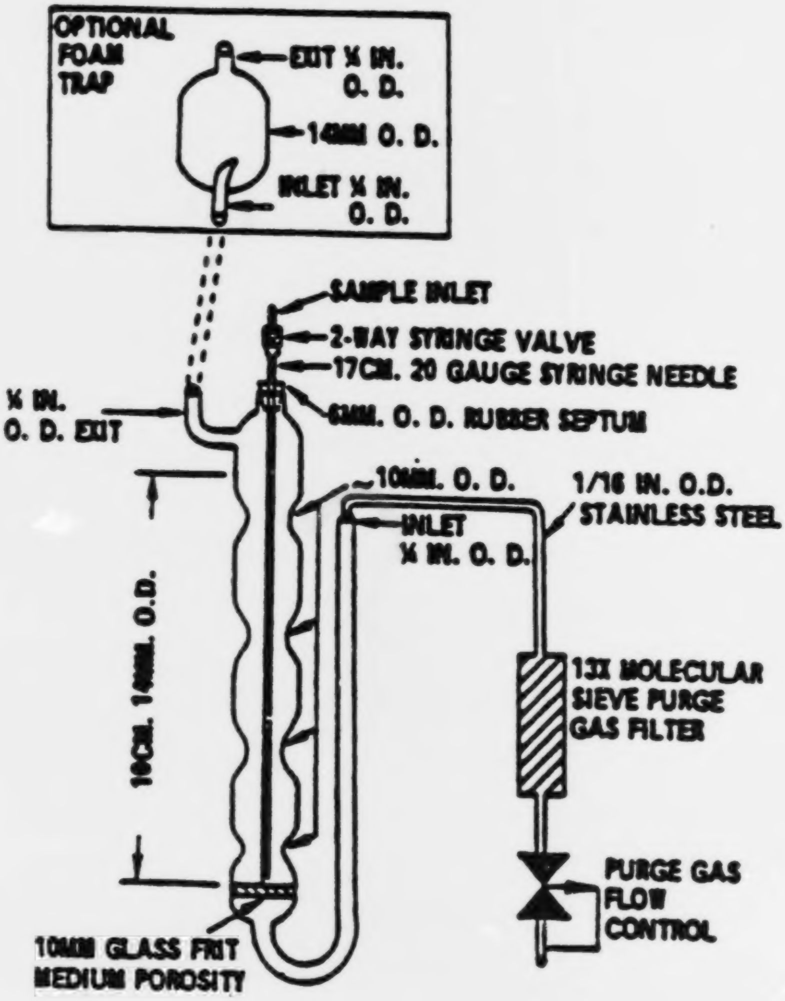
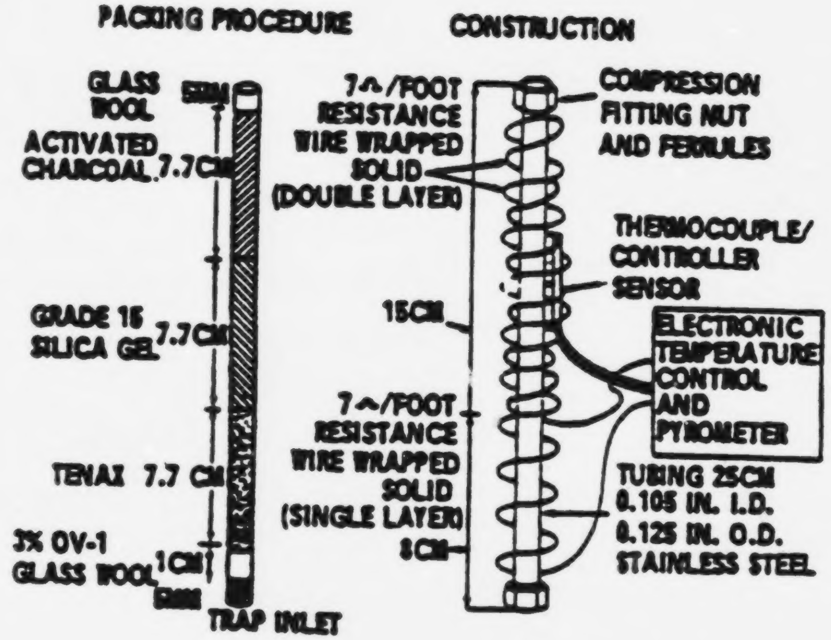

OPTIONAL FOAM TRAP
EXIT ¼ IN. O. D.
14MM O. D.
INLET ¼ IN. O. D.
SAMPLE INLET
2-WAY SYRINGE VALVE
17CM. 20 GAUGE SYRINGE NEEDLE
¼ IN. O. D. EXIT
6MM. O. D. RUBBER SEPTUM
10MM. O. D.
1/16 IN. O.D. STAINLESS STEEL
INLET ¼ IN. O. D.
10CM. 14MM. O.D.
13X MOLECULAR SIEVE PURGE GAS FILTER
PURGE GAS FLOW CONTROL
10MM GLASS FRIT MEDIUM POROSITY

FIGURE 1. PURGING DEVICE



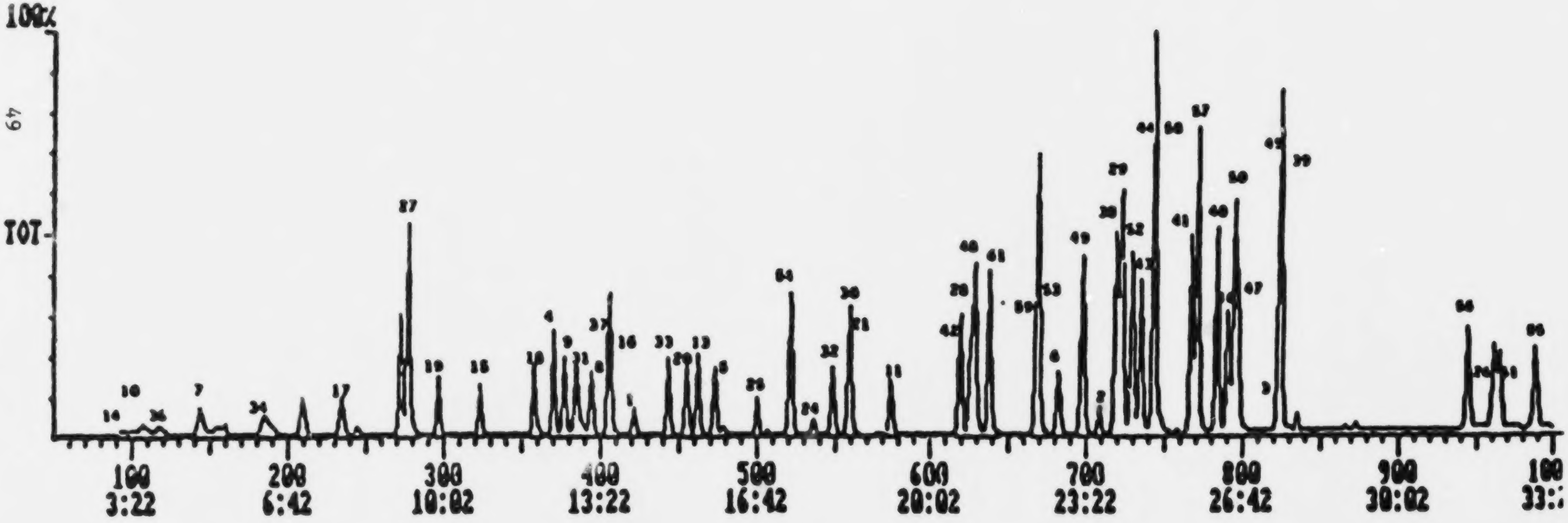
PACKING PROCEDURE

CONSTRUCTION

GLASS WOOL 5MM
ACTIVATED CHARCOAL 7.7CM
GRADE 15 SILICA GEL 7.7CM
TENAX 7.7 CM
3% OV-1 1CM
GLASS WOOL 5MM
TRAP INLET
7Ω/FOOT RESISTANCE WIRE WRAPPED SOLID (DOUBLE LAYER)
COMPRESSION FITTING NUT AND FERRULES
THERMOCOUPLE/ CONTROLLER SENSOR
15CM
ELECTRONIC TEMPERATURE CONTROL AND PYROMETER
7Ω/FOOT RESISTANCE WIRE WRAPPED SOLID (SINGLE LAYER)
8CM
TUBING 25CM 0.105 IN. I.D. 0.125 IN. O.D. STAINLESS STEEL

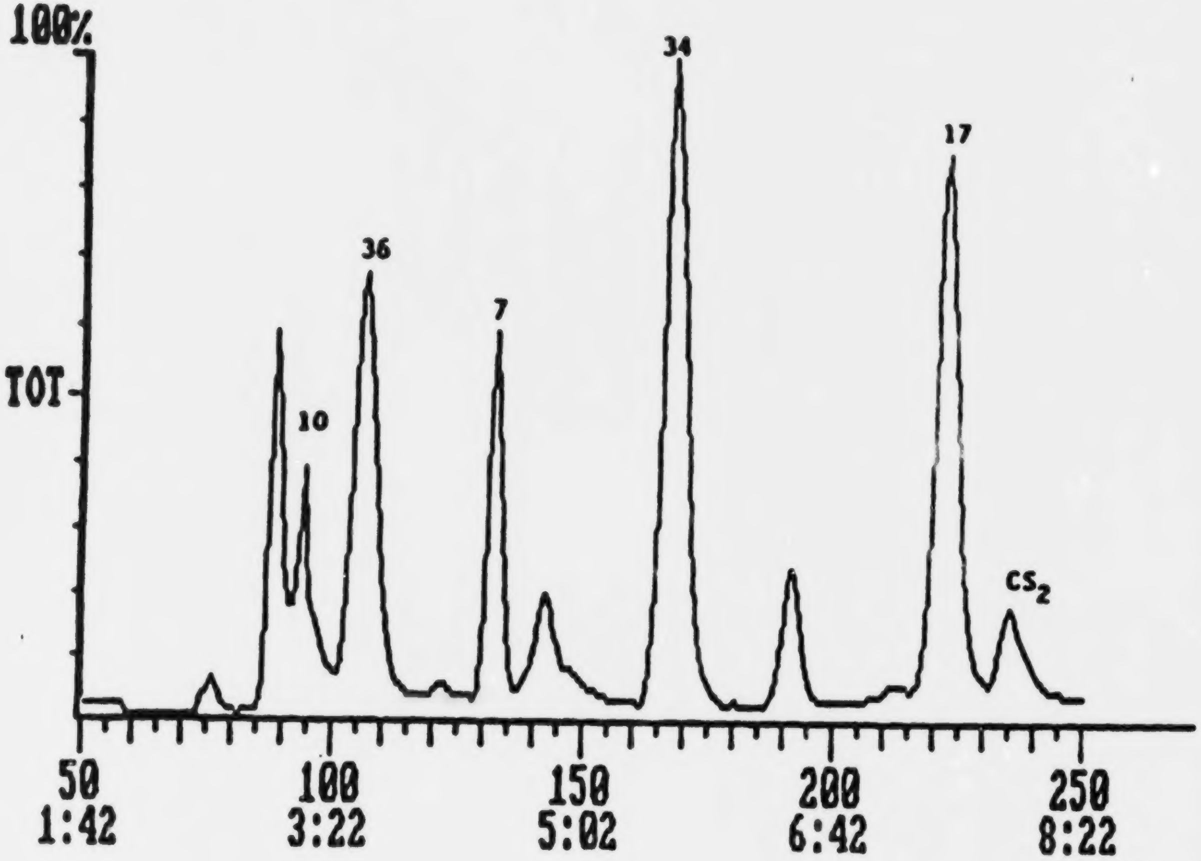
FIGURE 2. TRAP PACKINGS AND CONSTRUCTION TO INCLUDE DESORB CAPABILITY

FIGURE 3. NORMALIZED TOTAL ION CURRENT CHROMATOGRAM FROM A VOLATILE COMPOUND CALIBRATION MIXTURE CONTAINING 25 ng (5 µg/L) OF MOST COMPOUNDS. THE COMPOUND IDENTIFICATION NUMBERS ARE GIVEN IN TABLE 6.



Reproduced from
best available copy.

FIGURE 4. AMPLIFIED FIRST EIGHT MINUTES OF A TOTAL ION CURRENT CHROMATOGRAM FROM A VOLATILE COMPOUND CALIBRATION MIXTURE CONTAINING 25 ng (5 ug/L) OF EACH COMPONENT. THE COMPOUND IDENTIFICATION NUMBERS ARE GIVEN IN TABLE 6.



# METHOD 515.2. DETERMINATION OF CHLORINATED ACIDS IN WATER USING LIQUID-SOLID EXTRACTION AND GAS CHROMATOGRAPHY WITH AN ELECTRON CAPTURE DETECTOR

Revision 1.0

August 1992

R.C. Dressman and J.J. Lichtenberg - EPA 600/4-81-053, Revision 1.0 (1981)

J.W. Hodgeson - Method 515, Revision 2.0 (1986)

T. Engels (Battelle Columbus Laboratories) - National Pesticide Survey Method 3, Revision 3.0 (1987)

R.L. Graves - Method 515.1, Revision 4.0 (1989)

J.W. Hodgeson - Method 515.2, Revision 1.0 (1992)

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

# METHOD 515.2

## DETERMINATION OF CHLORINATED ACIDS IN WATER USING LIQUID-SOLID EXTRACTION AND GAS CHROMATOGRAPHY WITH AN ELECTRON CAPTURE DETECTOR

## 1. SCOPE AND APPLICATION

1.1 This is a gas chromatographic (GC) method applicable to the determination of certain chlorinated acids in ground water and finished drinking water. The following compounds can be determined by this method:

| Analyte | Chemical Abstract Services Registry Number |
|---|---|
| Acifluorfen | 50594-66-6 |
| Bentazon | 25057-89-0 |
| 2,4-D | 94-75-7 |
| 2,4-DB | 94-82-6 |
| Dacthal[a] | 1861-32-1 |
| Dicamba | 1918-00-9 |
| 3,5-Dichlorobenzoic acid | 51-36-5 |
| Dichlorprop | 120-36-5 |
| Dinoseb | 88-85-7 |
| 5-Hydroxydicamba | 7600-50-2 |
| Pentachlorophenol (PCP) | 87-86-5 |
| Picloram | 1918-02-1 |
| 2,4,5-T | 93-76-5 |
| 2,4,5-TP(Silvex) | 93-72-1 |

[a] Dacthal monoacid and diacid metabolites included in method scope; Dacthal diacid metabolite used for validation studies.

1.2 This method is applicable to the determination of salts and esters of analyte acids. The form of each acid is not distinguished by this method. Results are calculated and reported for each listed analyte as the total free acid.

1.3 Single laboratory accuracy and precision data and method detection limits (MDLs) have been determined for the analytes above (Sect. 13). Observed detection limits may vary among water matrices, depending upon the nature of interferences in the sample matrix and the specific instrumentation used.

1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of GC and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Sect. 9.3.

1.5 Analytes that are not separated chromatographically, (i.e., have very similar retention times) cannot be individually identified and measured in the same calibration mixture or water sample unless an alternative technique for identification and quantitation exists (Sect. 11.6).

1.6 When this method is used to analyze unfamiliar samples for any or all of the analytes above, analyte identifications should be confirmed by analysis on a second gas chromatographic column or by gas chromatography/mass spectrometry (GC/MS).

## 2. SUMMARY OF METHOD

2.1 A 250-mL measured volume of sample is adjusted to pH 12 with 6 N sodium hydroxide for 1 hr to hydrolyze derivatives. Extraneous organic material is removed by a solvent wash. The sample is acidified, and the chlorinated acids are extracted with a 47 mm resin based extraction disk. The acids are converted to their methyl esters using diazomethane. Excess derivatizing reagent is removed, and the esters are determined by capillary column GC using an electron capture detector (ECD).

## 3. DEFINITIONS

3.1 INTERNAL STANDARD (IS) -- A pure analyte(s) added to a sample, extract, or standard solution in known amount(s), and used to measure the relative responses of other method analytes and surrogates that are components of the same sample or solution. The IS must be an analyte that is not a sample component.

3.2 SURROGATE ANALYTE (SA) -- A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction or other processing, and is measured with the same procedures used to measure other sample components. The purpose of the SA is to monitor method performance with each sample.

3.3 LABORATORY DUPLICATES (LD1 AND LD2) -- Two aliquots of the same sample taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 FIELD DUPLICATES (FD1 AND FD2) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.5 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 FIELD REAGENT BLANK (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.7 INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.8 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.9 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot, and the measured values in the LFM corrected for background concentrations.

3.10 STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.11 PRIMARY DILUTION STANDARD SOLUTION (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions, and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.12 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.13 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

## 4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under analytical conditions by analyzing laboratory reagent blanks as described in Sect. 9.2.

4.1.1 Glassware must be scrupulously cleaned. (1) Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with dilute acid, tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 1 hr. Do not heat volumetric ware. Thermally stable materials such as PCBs might not be eliminated by this treatment. Thorough rinsing with acetone may be substituted for the heating. After glassware is dry and cool, store it in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

4.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required. WARNING: When a solvent is purified, stabilizers and preservatives added by the manufacturer are removed, thus potentially making the solvent hazardous and reducing the shelf life.

4.2 The acid forms of the analytes are strong organic acids which react readily with alkaline substances and can be lost during sample preparation. Glassware and glass wool must be acid-rinsed with 1 N hydrochloric acid and the sodium sulfate must be acidified with sulfuric acid prior to use to avoid analyte losses due to adsorption.

4.3 Organic acids and phenols, especially chlorinated compounds, cause the most direct interference with the determination. Alkaline hydrolysis and subsequent extraction of the basic sample removes many chlorinated hydrocarbons and phthalate esters that might otherwise interfere with the electron capture analysis.

4.4 Interferences by phthalate esters can pose a major problem in pesticide analysis when using the ECD. Phthalates generally appear in the chromatogram as large peaks. Common flexible plastics contain varying amounts of phthalates, that are easily extracted or leached during laboratory operations. Cross-contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Interferences from phthalates can best be minimized by avoiding the use of plastics in the laboratory. Exhaustive purification of reagents and glassware may be required to eliminate background phthalate contamination. (2,3)

4.5 Interfering contamination may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. Between-sample rinsing of the sample syringe and associated equipment with methyl-tert-butyl-ether (MTBE) can minimize sample cross-contamination. After analysis of a sample containing high concentrations of analytes, one or more injections of MTBE should be made to ensure that accurate values are obtained for the next sample.

4.6 Matrix interferences may be caused by contaminants that are coextracted from the sample. Also, note that all analytes listed in the Scope and Application Section are not resolved from each other on any one column, i.e., one analyte of interest may interfere with another analyte of interest. The extent of matrix interferences will vary considerably from source to source, depending upon the water sampled. The procedures in Sect. 11 can be used to overcome many of these interferences. Tentative identifications should be confirmed (Sect. 11.6).

4.7 It is important that samples and working standards be contained in the same solvent. The solvent for working standards must be the same as the final solvent used in sample preparation. If this is not the case, chromatographic comparability of standards to sample extracts may be affected.

## 5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. Accordingly, exposure to these chemicals must be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified (5-7) for the information of the analyst.

5.2 DIAZOMETHANE -- A toxic carcinogen which can explode under certain conditions. The following precautions must be followed:

5.2.1 Use the diazomethane generator behind a safety shield in a well ventilated fume hood. Under no circumstances can the generator be heated above 90°C, and all grinding surfaces such as ground glass joints, sleeve bearings, and glass stirrers must be avoided. Diazomethane solutions must not be stored. Only generate enough for the immediate needs. The diazomethane generator apparatus used in the esterification procedure (Sect. 11.4) produces micromolar amounts of diazomethane in solution to minimize safety hazards. If the procedure is followed exactly, no possibility for explosion exists.

5.3 METHYL-TERT-BUTYL ETHER -- Nanograde, redistilled in glass, if necessary. Must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Cat. No. P1126-8, and other suppliers).

5.4 WARNING: When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous.

6. **EQUIPMENT AND SUPPLIES** (All specifications are suggested. Catalog numbers are included for illustration only.)

6.1 KONTES FILTER FUNNELS -- Fisher Cat. No. 953755-0000 or equivalent.

6.2 VACUUM FLASKS -- 1000 mL with glass side arm

6.3 VACUUM MANIFOLD -- The manifold should be capable of holding 6-8 filter flasks in series with house vacuum. Commercial manifolds are available from a number of suppliers, e.g., Baker, Fisher, and Varian.

6.4 CULTURE TUBES (25 x 200 mm) WITH TEFLON-LINED SCREW CAPS -- Fisher Cat. No. 14-933-1C, or equivalent.

6.5 PASTEUR PIPETS -- Glass disposable (5 mL)

6.6 LARGE VOLUME PIPETS -- Disposable, Fisher Cat. No. 13-678-8 or equivalent.

6.7 BALANCE -- Analytical, capable of weighing to .0001 g.

6.8 pH METER -- Wide range capable of accurate measurements in the pH = 1-12 range.

6.9 DIAZOMETHANE GENERATOR -- See Figure 1 for a diagram of an all glass system custom made for these validation studies. A micromolar generator is also available from Aldrich Chemical.

6.10 ANALYTICAL CONCENTRATOR -- Six or twelve positions, Organomation N-EVAP Model No. 111-6917 or equivalent.

6.11 GAS CHROMATOGRAPHY -- Analytical system complete with gas chromatograph equipped with ECD, split/splitless capillary injector, temperature programming, differential flow control and all required accessories. A data system is recommended for measuring peak areas. An autoinjector is recommended to improve precision of analysis.

6.12 GC COLUMNS AND RECOMMENDED OPERATING CONDITIONS

6.12.1 Primary -- DB-5 or equivalent, 30 m x .32 mm ID, 0.25 µm film thickness. Injector Temp. = 200°C, Detector Temp. = 280°C, Helium linear velocity is 30 cm/sec at 200°C and 10 psi, 2 µL splitless injection with purge on 3 min. Program: Hold at 60°C 1 min., increase to 260°C at 5°C/min. and hold 5 min.

6.12.2 Confirmation -- DB-1701 or equivalent, 30 m x .32 mm ID, 0.25 µm film thickness. Injector Temp. = 200 °C, Detector Temp. = 280°C, Helium linear velocity is 30 cm/sec at 200°C and 10 psi, 2 µL splitless injection with purge on 3 min. Program: Hold at 60°C 1 min., increase to 260°C at 5°C/min. and hold 5 min.

6.13 GLASS WOOL -- Acid washed with 1N HCl and heated at 450°C for 4 hr.

6.14 SHORT RANGE pH PAPER (pH=0-3).

6.15 VOLUMETRIC FLASKS -- 50 mL, 100 mL, and 250 mL

6.16 MICROSYRINGES -- 25 µL, 50 µL, 100 µL, 250 µL, 500 µL

6.17 AMBER BOTTLES -- 15 mL, with Teflon-lined screw caps

6.18 GRADUATED CYLINDER -- 250 mL

6.19 SEPARATORY FUNNEL -- 500 mL

6.20 GRADUATED CENTRIFUGE TUBES -- 15 mL or 10 mL Kuderna Danish Concentrator tubes

## 7. REAGENTS AND STANDARDS

7.1 EXTRACTION DISKS, 47 mm -- Resin based polystyrenedivinylbenzene

7.2 REAGENT WATER -- Reagent water is defined as a water in which an interference is not observed at the MDL of each analyte of interest.

7.2.1 A Millipore Super-Q water system or its equivalent may be used to generate deionized reagent water. Distilled water that has been passed through granular charcoal may also be suitable.

7.2.2 Test reagent water each day it is used by analyzing according to Sect. 11.

7.3 METHANOL -- Pesticide quality or equivalent.

7.4 METHYL-TERT-BUTYL ETHER (MTBE) -- Nanograde, redistilled in glass if necessary. Ether must be demonstrated to be free of peroxides. One test kit (EM Quant Test Strips), is available from EM Science, Gibbstown, NJ. Procedures for removing peroxides from the ether are provided with the test strips. Ethers must be periodically tested (at least monthly) for peroxide formation during use. Any reliable test kit may be used.

7.5 SODIUM SULFATE -- (ACS) GRANULAR, ANHYDROUS -- Heat in a shallow tray at 400°C for a minimum of 4 hr to remove phthalates and other interfering organic substances. Alternatively, extract with methylene chloride in a Soxhlet apparatus for 48 hr.

7.5.1 Sodium sulfate drying tubes -- Plug the bottom of a large volume disposable pipet with a minimum amount of acidified glass wool (Supelco Cat. No. 20383 or equivalent). Fill the pipet halfway (3 g) with acidified sodium sulfate (See Sect. 7.9).

7.6 SULFURIC ACID -- Reagent grade.

7.6.1 Sulfuric acid, 12 N -- Slowly add 335 mL concentrated sulfuric acid to 665 mL of reagent water.

7.7 SODIUM HYDROXIDE -- ACS reagent grade or equivalent.

7.7.1 Sodium hydroxide 1N -- Dissolve 4.0 g reagent grade sodium hydroxide in reagent water and dilute to 100 mL in volumetric flasks.

7.7.2 Sodium hydroxide 6N

7.8 ETHYL ETHER, UNPRESERVED -- Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Cat. No. PI126-8, and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips.

7.9 ACIDIFIED SODIUM SULFATE -- Cover 500 g sodium sulfate (Sect. 7.5) with ethyl ether (Sect. 7.8). While agitating vigorously, add dropwise approximately 0.7 mL concentrated sulfuric acid. Remove the ethyl ether overnight under vacuum and store the sodium sulfate in a 100°C oven.

7.10 CARBITOL, ACS GRADE -- Available from Aldrich Chemical.

7.11 DIAZALD, ACS GRADE -- Available from Aldrich Chemical.

7.12 DIAZALD SOLUTION -- Prepare a solution containing 10 g Diazald in 100 mL of a 50:50 by volume mixture of ethyl ether and carbitol.

This solution is stable for 1-month or longer when stored at 4°C in an amber bottle with a Teflon-lined screw cap.

7.13 4,4'-DIBROMOOCTAFLUOROBIPHENYL (DBOB) -- 99% purity, for use as internal standard.

7.14 2,4-DICHLOROPHENYLACETIC ACID (DCAA) -- 99% purity, for use as surrogate standard.

7.15 POTASSIUM HYDROXIDE -- ACS reagent grade or equivalent.

7.15.1 Potassium hydroxide solution, 37% -- Using extreme caution, dissolve 37 g reagent grade potassium hydroxide in reagent water and dilute to 100 mL.

7.16 STOCK STANDARD SOLUTIONS (1.00-2.00 µg/µL) -- Stock standard solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedure:

7.16.1 Prepare stock standard solutions by accurately weighing approximately 0.0100-0.0200 g of pure material. Dissolve the material in methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

7.16.2 Transfer the stock standard solutions into 15-mL TFE-fluorocarbon-sealed screw cap amber vials. Store at 4°C or less when not in use.

7.16.3 Stock standard solutions should be replaced after 2 months or sooner if comparison with laboratory fortified blanks, or QC samples indicate a problem.

7.16.4 Primary Dilution Standards -- Prepare two sets of standards according to the sets labeled A and B in Table 1. For each set, add approximately 25 mL of methanol to a 50 mL volumetric flask. Add aliquots of each stock standard in the range of approximately 20 to 400 µL and dilute to volume with methanol. Individual analyte concentrations will then be in the range of 0.4 to 8 µg/mL (for a 1.0 mg/mL stock). The minimum concentration would be appropriate for an analyte with strong electron capture detector (ECD) response, e.g. pentachlorophenol. The maximum concentration is for an analyte with weak response, e.g., 2,4-DB. The concentrations given in Table 2 reflect the relative volumes of stock standards used for the primary dilution standards used in generating the method validation data. Use these relative values to determine the aliquot volumes of individual stock standards above.

7.17 INTERNAL STANDARD SOLUTION -- Prepare a stock internal standard solution by accurately weighing approximately 0.050 g of pure DBOB. Dissolve the DBOB in methanol and dilute to volume in a 10-mL volumetric flask. Transfer the DBOB solution to a TFE-fluorocarbon-sealed screw cap bottle and store at room temperature. Prepare a primary dilution standard at approximately 1.00 µg/mL by the addition of 20 µL of the stock standard to 100 mL of methanol. Addition of 100 µL of the primary dilution standard solution to the final 5 mL of sample extract (Sect. 11) results in a final internal standard concentration of 0.020 µg/mL. Solution should be replaced when ongoing QC (Sect. 9) indicates a problem. Note that DBOB has been shown to be an effective internal standard for the method analytes, but other compounds may be used if the QC requirements in Sect. 9 are met.

7.18 SURROGATE ANALYTE SOLUTION -- Prepare a surrogate analyte stock standard solution by accurately weighing approximately 0.050 g of pure DCAA. Dissolve the DCAA in methanol and dilute to volume in a 10-mL volumetric flask. Transfer the surrogate analyte solution to a TFE-fluorocarbon-sealed screw cap bottle and store at room temperature. Prepare a primary dilution standard at approximately 2.0 µg/mL by addition of 40 µL at the stock standard to 100 mL of methanol. Addition of 250 µL of the surrogate analyte solution to a 250-mL sample prior to extraction results in a surrogate concentration in the sample of 2 µg/L and, assuming quantitative recovery of DCAA, a surrogate analyte concentration in the final 5 mL extract of 0.1 µg/mL. The surrogate standard solution should be replaced when ongoing QC (Sect. 9) indicates a problem. DCAA has been shown to be an effective surrogate standard for the method analytes, but other compounds may be used if the QC requirements in Sect. 10 are met.

7.19 INSTRUMENT PERFORMANCE CHECK SOLUTION -- Prepare a diluted dinoseb solution by adding 10 µL of the 1.0 µg/µL dinoseb stock solution to the MTBE and diluting to volume in a 10-mL volumetric flask. To prepare the check solution, add 40 µL of the diluted dinoseb solution, 16 µL of the 4-nitrophenol stock solution, 6 µL of the 3,5-dichlorobenzoic acid stock solution, 50 µL of the surrogate standard solution, 25 µL of the internal standard solution, and 250 µL of methanol to a 5-mL volumetric flask and dilute to volume with MTBE. Methylate sample as described in Sect. 11.4. Dilute the sample to 10 mL in MTBE. Transfer to a TFE-fluorocarbon-sealed screw cap bottle and store at room temperature. Solution should be replaced when ongoing QC (Sect. 9) indicates a problem.

## 8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Grab samples should be collected in 1-L amber glass containers. Conventional sampling practices (7) should be followed; however, the bottle must not be prerinsed with sample before collection.

## 8.2 SAMPLE PRESERVATION AND STORAGE

8.2.1 Add hydrochloric acid (diluted 1:1 in water) to the sample at the sampling site in amounts to produce a sample pH $\leq 2$. Short range (0-3) pH paper (Sect. 6.14) may be used to monitor the pH.

8.2.2 If residual chlorine is present, add 80 mg of sodium thiosulfate per liter of sample to the sample bottle prior to collecting the sample.

8.2.3 After the sample is collected in the bottle containing preservative(s), seal the bottle and shake vigorously for 1 min.

8.2.4 The samples must be iced or refrigerated at 4°C away from light from the time of collection until extraction. Preservation study results indicate that the sample analytes (measured as total acid), except 5-hydroxy-dicamba, are stable in water for 14 days when stored under these conditions (Tables 8 and 9). The concentration of 5-hydroxydicamba is seriously degraded over 14 days in a biologically active matrix. However, analyte stability will very likely be affected by the matrix; therefore, the analyst should verify that the preservation technique is applicable to the samples under study.

## 8.3 EXTRACT STORAGE

8.3.1 Extracts should be stored at 4°C or less away from light. Preservation study results indicate that most analytes are stable for 14 days (Tables 8 and 9); however, the analyst should verify appropriate extract holding times applicable to the samples under study.

# 9. QUALITY CONTROL

9.1 Minimum QC requirements are initial demonstration of laboratory capability, determination of surrogate compound recoveries in each sample and blank, monitoring internal standard peak area or height in each sample and blank (when internal standard calibration procedures are being employed), analysis of laboratory reagent blanks, laboratory fortified samples, laboratory fortified blanks, and QC samples.

9.2 LABORATORY REAGENT BLANKS (LRB) -- Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window of any analyte the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

## 9.3 INITIAL DEMONSTRATION OF CAPABILITY

9.3.1 Select a representative fortified concentration (about 10 to 20 times MDL) for each analyte. Prepare a sample concentrate (in methanol) containing each analyte at 1000 times selected concentration. With a syringe, add 250 µL of the concentrate to each of at least four 250 mL aliquots of reagent water, and analyze each aliquot according to procedures beginning in Sect. 11.

9.3.2 For each analyte the recovery value for all four of these samples must fall in the range of ± 40% of the fortified concentration. For those compounds that meet the acceptance criteria, performance is considered acceptable and sample analysis may begin. For compounds failing this criteria, this procedure must be repeated using five fresh samples until satisfactory performance has been demonstrated for all analytes.

9.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. As laboratory personnel gain experience with this method the quality of data should improve beyond those required here.

9.4 The analyst is permitted to modify GC columns, GC conditions, detectors, concentration techniques (i.e., evaporation techniques), internal standard or surrogate compounds. Each time such method modifications are made, the analyst must repeat the procedures in Sect. 9.3.

## 9.5 ASSESSING SURROGATE RECOVERY

9.5.1 When surrogate recovery from a sample or a blank is <60% or > 140%, check (1) calculations to locate possible errors, (2) fortifying solutions for degradation, (3) contamination, and (4) instrument performance. If those steps do not reveal the cause of the problem, reanalyze the extract.

9.5.2 If a blank extract reanalysis fails the 60-140% recovery criteria, the problem must be identified and corrected before continuing.

9.5.3 If sample extract reanalysis meets the surrogate recovery criteria, report only data for the reanalyzed extract. If sample extract continues to fail the recovery criteria, report all data for that sample as suspect.

## 9.6 ASSESSING THE INTERNAL STANDARD

9.6.1 When using the internal standard (IS) calibration procedure, the analyst is expected to monitor the IS response (peak area or peak height) of all samples during each analysis day. The IS response for any sample chromatogram should not deviate from the daily calibration check standard's IS response by more than 30%.

9.6.2 If >30% deviation occurs with an individual extract, optimize instrument performance and inject a second aliquot of that extract.

9.6.2.1 If the reinjected aliquot produces an acceptable internal standard response, report results for that aliquot.

9.6.2.2 If a deviation of greater than 30% is obtained for the reinjected extract, analysis of the samples should be repeated beginning with Sect. 11, provided the sample is still available. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.

9.6.3 If consecutive samples fail the IS response acceptance criteria, immediately analyze a medium calibration standard.

9.6.3.1 If the standard provides a response factor (RF) (Sect. 10.2.2) within 20% of the predicted value, then follow procedures itemized in Sect. 9.6.2 for each sample failing the IS response criterion.

9.6.3.2 If the check standard provides a response factor which deviates more than 20% of the predicted value, then the analyst must recalibrate as specified in Sect. 10.

## 9.7 ASSESSING LABORATORY PERFORMANCE -- LABORATORY FORTIFIED BLANK

9.7.1 The laboratory must analyze at least one laboratory fortified blank (LFB) sample with every 20 samples or one per sample set (all samples extracted within a 24-hr period) whichever is greater. The concentration of each analyte in the LFB should be 10 times the MDL. Calculate percent recovery ($X_i$). If the recovery of any analyte falls outside the control limits (See Sect. 9.7.2), that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.7.2 Until sufficient data become available, usually a minimum of results from 20 to 30 analyses, each laboratory should assess laboratory performance against the control limits in Sect. 9.3.2 that are derived from the data in Table 2. When sufficient internal performance data become available, develop control limits from the mean percent recovery (X) and standard deviation (S) of the percent recovery. These data are used to establish upper and lower control limits as follows:

UPPER CONTROL LIMIT = X + 3S
LOWER CONTROL LIMIT = X - 3S

After each five to ten new recovery measurements, new control limits should be calculated using only the most recent 20-30 data points. These calculated control limits should never exceed those established in Sect. 9.3.2.

9.7.3 Method detection limits (MDL) must be determined using the procedure given in reference (8). The MDLs must be sufficient to detect analytes at the required levels according to SDWA regulations.

9.7.4 At least quarterly, analyze a QCS (Sect. 3.13) from an outside source.

9.7.5 Laboratories are encouraged to participate in external performance evaluation studies such as the laboratory certification programs offered by many states or the studies conducted by USEPA.

### 9.8 ASSESSING ANALYTE RECOVERY - LABORATORY FORTIFIED SAMPLE MATRIX

9.8.1 Each laboratory must analyze a LFM for 10% of the samples or one sample concentration per set, whichever is greater. The concentration should not be less then the background concentration of the sample selected for fortification. Ideally, the concentration should be the same as that used for the laboratory fortified blank (Sect. 9.7). Over time, samples from all routine sample sources should be fortified.

9.8.2 Calculate the percent recovery, P of the concentration for each analyte, after correcting the measured concentration, X, from the fortified sample for the background concentration, b, measured in the unfortified sample.

$$P = 100\ (X - b)\ /\ \text{fortified concentration},$$

and compare these values to control limits appropriate for reagent water data collected in the same fashion. If the analyzed unfortified sample is found to contain **NO** background concentrations and the added concentrations are those specified in Sect. 9.7, then the appropriate control limits would be the acceptance limits in Sect. 9.7. If, on the other hand, the analyzed unfortified sample is found to contain background concentration, b, estimate the standard deviation at the background concentration, $s_b$, using regressions or comparable background data and, similarly, estimate the mean, $X_a$ and standard deviation, $s_a$, of analytical results at the total concentration after fortifying. Then the appropriate percentage control limits would be $P \pm 3s_p$ , where:

$$\overline{P} = 100\ \overline{X}\ /\ (b + \text{fortifying concentration})$$

$$\text{and } s_P = 100\ (s_a^2 + s_b^2)^{1/2}\ /\text{fortifying concentration}$$

For example, if the background concentration for Analyte A was found to be 1 μg/L and the added amount was also 1 μg/L, and upon analysis the laboratory fortified sample measured 1.6 μg/L, then the calculated P for this sample would be (1.6 μg/L minus 1.0 μg/L) /1 μg/L or 60%. This calculated P is compared to control limits derived from prior reagent water data. Assume that analysis of an interference free sample at 1 μg/L yields_an s of 0.12 μg/L and similar analysis at 2.0 μg/L yields $\overline{X}$ and s of 2.01 μg/L and 0.20 μg/L, respectively. The appropriate limits to judge the reasonableness of the percent recovery, 60%, obtained on the fortified matrix sample is computed as follows:

$$[100\ (2.01\ \mu g/L)\ /\ 2.0\ \mu g/L]$$

$$\pm\ 3\ (100)\ \left[(0.12\ \mu g/L)^2 + (0.20\ \mu g/L)^2\right]^{1/2}\ /\ 1.0\ \mu g/L =$$

$$100.5\% \pm 300\ (0.233) =$$

100.5% ± 70% or 30% to 170% recovery of the added analyte.

9.8.3 If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Sect. 9.7), the recovery problem encountered with the fortified sample is judged to be matrix related, not system related. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.9 ASSESSING INSTRUMENT SYSTEM/INSTRUMENT PERFORMANCE CHECK (IPC) SAMPLE -- Instrument performance should be monitored on a daily basis by analysis of the IPC sample. The IPC sample contains compounds designed to indicate appropriate instrument sensitivity, column performance (primary column) and chromatographic performance. IPC sample components and performance criteria are listed in Table 11. Inability to demonstrate acceptable instrument performance indicates the need for reevaluation of the instrument system. The sensitivity requirements are set based on the MDLs published in this method. MDLs will vary from laboratory to laboratory.

9.10 The laboratory may adopt additional QC practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation, and storage.

## 10. CALIBRATION AND STANDARDIZATION

10.1 Establish GC operating parameters equivalent to those indicated in Sect. 6.12. This calibration procedure employs procedural standards, i.e., fortified aqueous standards which are processed through most of the method (Sect. 11). The GC system is calibrated by means of the internal standard technique (Sect. 10.2). NOTE: Calibration standard solutions must be prepared such that no unresolved analytes are mixed together (See Table 1).

10.2 INTERNAL STANDARD CALIBRATION PROCEDURE -- To use this approach, the analyst must select one or more internal standards compatible in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. DBOB (Sect. 7.13) has been identified as a suitable internal standard.

10.2.1 Prepare aqueous calibration standards at a minimum of three (five are recommended) concentration levels for each method analyte as follows: for each concentration, fill a 250-mL volumetric flask with 240 mL of reagent water at pH 1 and containing 20% by weight of dissolved sodium sulfate. Add an appropriate aliquot of the primary dilution standard (Sect. 7.16.4) and dilute to 250 mL with the same reagent water. Process each aqueous calibration sample through the analytical procedure beginning with Sect. 11.2, i.e., omit the hydrolysis and cleanup step (Sect. 11.1). The lowest calibration standard should represent analyte concentrations near, but above, the respective MDLs. The remaining standards should bracket the analyte concentrations expected in the sample extracts, or should define the working range of the detector. The internal standard is added to the final 5 mL extract as specified in Sect. 11.

10.2.2 Analyze each calibration standard according to the procedure beginning in Sect. 11.2. Tabulate response (peak height or area) against concentration for each compound and internal standard. Calculate the response factor (RF) for each analyte and surrogate using Equation 1.

$$RF = \frac{(A_s)\ (C_{is})}{(A_{is})\ (C_s)} \qquad \text{Equation 1}$$

where:

$A_s$ = Response for the analyte to be measured.
$A_{is}$ = Response for the internal standard.
$C_{is}$ = Concentration of the internal standard ($\mu g/L$).
$C_s$ = Concentration of the analyte to be measured ($\mu g/L$).

10.2.3 If the RF value over the working range is constant (30% RSD or less) the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios ($A_s/A_{is}$) vs. $C_s$. A data station may be used to collect the chromatographic data, calculate response factors and generate linear or second order regression curves.

10.2.4 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. A new calibration standard need not be derivatized each day. The same standard extract can be used up to 14 days. If the response for any analyte varies from the predicted response by more than ±30%, the test must be repeated using a fresh calibration standard. If the repetition also fails, a new calibration curve must be generated for that analyte using freshly prepared standards.

10.2.5 Verify calibration standards periodically, at least quarterly is recommended, by analyzing a standard prepared from reference material obtained from an independent source. Results from these analyses must be within the limits used to routinely check calibration.

## 11. PROCEDURE

### 11.1 MANUAL HYDROLYSIS AND CLEAN-UP

11.1.1 Remove the sample bottles from cold storage and allow them to equilibrate to room temperature. Acidify and add sodium thiosulfate to blanks and QC check standards as specified in Sect. 8.

11.1.2 Measure a 250-mL aliquot of each sample with a 250-mL graduated cylinder and pour into a 500-mL separatory funnel. Add 250 µL of the surrogate primary dilution standard (Sect. 7.18) to each 250-mL sample. The surrogate will be at a concentration of 2 µg/L. Dissolve 50 g sodium sulfate in the sample.

11.1.3 Add 4 mL of 6 N NaOH to each sample, seal, and shake. Check the pH of the sample with pH paper or a pH meter; if the sample does not have a pH greater than or equal to 12, adjust the pH by adding more 6 N NaOH. Let the sample sit at room temperature for 1 hr, shaking the separatory funnel and contents periodically.

11.1.4 Add 15 mL methylene chloride to the graduated cylinder to rinse the walls, transfer the methylene chloride to the separatory funnel and extract the sample by vigorously shaking the funnel for 2 min with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration through glass wool, centrifugation, or other physical methods. Discard the methylene chloride phase.

11.1.5 Add a second 15-mL volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time, discarding the methylene chloride layer. Perform a third extraction in the same manner.

11.1.6 Drain the contents of the separatory funnel into a 500-mL beaker. Adjust the pH to 1.0 ± 0.1 by the dropwise addition of concentrated sulfuric acid with constant stirring. Monitor the pH with a pH meter (Sect. 6.8) or short range (0-3) pH paper (Sect. 6.14).

## 11.2 SAMPLE EXTRACTION

11.2.1 Vacuum Manifold -- Assemble a manifold (Sect. 6.3) consisting of 6-8 vacuum flasks with filter funnels (Sect. 6.1,6.2). Individual vacuum control, on-off and vacuum release valves and vacuum gauges are desirable. Place the 47 mm extraction disks (Sect. 7.1) on the filter frits.

11.2.2 Add 20 mL of 10% by volume of methanol in MTBE to the top of each disk without vacuum and allow the solvent to remain for 2 min. Turn on full vacuum and pull the solvent through the disks, followed by room air for 5 min.

11.2.3 Adjust the vacuum to approximately 5 in. (mercury) and add the following in series to the filter funnel (a) 20 mL methanol (b) 20 mL reagent water (c) sample. Do not allow the disk to dry between steps and maintain the vacuum at 5 in.

11.2.4 After the sample is extracted completely, apply maximum vacuum and draw room air through the disks for 20 min.

11.2.5 Place the culture tubes (Sect. 6.4) in the vacuum tubes to collect the eluates. Elute the disks with two each 2-mL aliquots of 10% methanol in MTBE. Allow each aliquot to remain on the disk for one min before applying vacuum.

11.2.6 Rinse each 500-mL beaker (Sect.11.1.6) with 4 mL of pure MTBE and elute the disk with this solvent as in Sect. 11.2.5.

11.2.7 Remove the culture tubes and cap.

## 11.3 EXTRACT PREPARATION

11.3.1 Pre-rinse the drying tubes (Sect. 7.5.1) with 2 mL of MTBE.

11.3.2 Remove the entire extract with a 5-mL pipet and drain the lower aqueous layer back into the culture tube. Add the organic layer to the sodium sulfate drying tube (Sect. 7.5.1). Maintain liquid in the drying tube between this and subsequent steps. Collect the dried extract in a 15-mL graduated centrifuge tube or a 10-mL Kuderna-Danish tube.

11.3.3 Rinse the culture tube with an additional 1 mL of MTBE and repeat Sect. 11.3.2.

11.3.4 Repeat step Sect. 11.3.3 and finally add a 1-mL aliquot of MTBE to the drying tube before it empties. The final volume should be 6-9 mL. In this form the extract is esterified as described below.

## 11.4 EXTRACT ESTERIFICATION

11.4.1 Assemble the diazomethane generator (Figure 1) in a hood.

11.4.2 Add 5 mL of ethyl ether to Tube 1. Add 4 mL of Diazald solution (Sect. 7.12) and 3 mL of 37% KOH solution (Sect. 7.15.1) to the reaction tube 2. Immediately place the exit tube into the collection tube containing the sample extract. Apply nitrogen flow (10 mL/min) to bubble diazomethane through the extract. Each charge of the generator should be sufficient to esterify four samples. The appearance of a persistent yellow color is an indication that esterification is complete. The first sample should require 30 sec to 1 min and each subsequent sample somewhat longer. The final sample may require 2-3 min.

11.4.3 Cap each collection tube and allow to remain stored at room temperature in a hood for 30 min. No significant fading of the yellow color should occur during this period. Fortify each sample with 100 µL of the internal standard primary dilution solution (Sect. 7.17) and reduce the volume to 5.0 mL with the analytical concentrator (Sect. 6.10), a stream of dry nitrogen, or an equivalent concentration technique. NOTE: The excess diazomethane is volatilized from the extract during the concentration procedure.

11.4.4 Cap the tubes and store in a refrigerator if further processing will not be performed immediately. Analyze by GC-ECD.

## 11.5 GAS CHROMATOGRAPHY

11.5.1 Sect. 6.12 summarizes the recommended GC operating conditions. Included in Table 1 are retention times observed using this method. Figures 2A and 2B illustrate the chromatographic performance of the primary column (Sect. 6.12.1) for groups A and B of the method analytes. Other GC columns, chromatographic conditions, or detectors may be used if the requirements of Sect. 9.3 are met.

11.5.2 Calibrate the system daily as described in Sect. 10.

11.5.3 Inject 2 µL of the sample extract. Record the resulting peak size in area units.

11.5.4 If the response for any sample peak exceeds the working range of the detector, dilute the extract and reanalyze.

## 11.6 IDENTIFICATION OF ANALYTES

11.6.1 Identify a sample component by comparison of its retention time to the retention time of a reference chromatogram. If the retention time of an unknown compound corresponds, within limits, to the retention time of a standard compound, then an analyte is considered to be identified.

11.6.2 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in interpretation of chromatograms.

11.6.3 Identification requires expert judgment when sample components are not resolved chromatographically. When GC peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima, or any time doubt exists over the identification of a peak in a chromatogram, appropriate alternative techniques to help confirm peak identification need to be employed. For example, more positive identification may be made by the use of an alternative detector which operates on a chemical/physical principle different from that originally used, e.g., mass spectrometry, or the use of a second chromatography column. A suggested alternative column is described in Sect. 6.12.2.

## 12. DATA ANALYSIS AND CALCULATIONS

12.1 Calculate analyte concentrations in the sample from the response for the analyte using the calibration procedure described in Sect. 10.

12.2 Calculate the concentration (C) in the sample using the response factor (RF) determined in Sect. 10.2.2 and Equation 2, or determine sample concentration from the calibration curve (Sect. 10.2.3).

$$C\ (\mu g/L) = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)} \qquad \text{Equation 2.}$$

where:

$A_s$ = Response for the analyte to be measured.
$A_{is}$ = Response for the internal standard.
$I_s$ = Amount of internal standard added to each extract ($\mu g$).
$V_o$ = Volume of water extracted (L).

## 13. METHOD PERFORMANCE

13.1 In a single laboratory, analyte recoveries from reagent water were determined at three concentration levels, Tables 2-4. Results were used to determine the analyte MDLs (8) listed in Table 2.

13.2 In a single laboratory, analyte recoveries from dechlorinated tap water were determined at two concentrations, Tables 5 and 6. In addition, analyte recoveries were determined at two concentrations from an ozonated surface (river) water, Tables 7 and 8, and at one level from a high humectant surface (reservoir) water, Table 10. Finally, a holding study was conducted on the preserved, ozonated surface water and recovery data are presented for day 1 and day 14 of this study, Tables 8 and 9. The ozonated surface water was chosen as the matrix in which to study analyte stability during a 14-day holding time because it was very biologically active.

## 14. POLLUTION PREVENTION

14.1 This method utilizes the new liquid-solid extraction technology which requires the use of very small quantities of organic solvents. This feature eliminates the hazards involved with the use of large volumes of potentially harmful organic solvents needed for conventional liquid-liquid extractions. Also, mercuric chloride, a highly toxic and environmentally hazardous chemical, has been replaced with hydrochloric acid as the sample preservative. These features make this method much safer and a great deal less harmful to the environment. Some of the phenolic herbicides on the analyte list are very difficult to methylate and diazomethane is still required to derivatize these compounds.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

## 15. WASTE MANAGEMENT

15.1 Due to the nature of this method, there is little need for waste management. No large volumes of solvents or hazardous chemicals are used. The matrices of concern are finished drinking water or source water. However, the Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel," also available from the American Chemical Society at the address in Sect. 14.2.

## 16. REFERENCES

1. ASTM Annual Book of Standards, Part 11, Volume 11.02, D3694-82, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 86, 1986.

2. Giam, C.S., H.S. Chan, and G.S. Nef. "Sensitive Method for Determination of Phthalate Ester Plasticizers in Open-Ocean Biota Samples," Analytical Chemistry, 47, 2225 (1975).

3. Giam, C.S., and H.S. Chan. "Control of Blanks in the Analysis of Phthalates in Air and Ocean Biota Samples." U.S. National Bureau of Standards, Special Publication 442, pp. 701-708, 1976.

4. "Carcinogens - Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.

5. "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).

6. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

7. ASTM Annual Book of Standards, Part 11, Volume 11.01, D3370-82, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 130, 1986.

8. Glaser, J.A., Foerst, D.L., McKee, G.D., Quave, S.A., and Budde, W.L., "Trace Analyses for Wastewaters," *Environ. Sci. Technol.* 1981, *15*, 1426-1435.

9. 40 CFR, Part 136, Appendix B.

## 17. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

### TABLE 1. RETENTION DATA

| | | Retention Time, min.[b] | |
|---|---|---|---|
| Analyte | Group[a] | Primary | Confirmation |
| 3,5-Dichlorobenzoic acid | A | 16.72 | 18.98 |
| 2,4-Dichlorophenylacetic acid (SA) | A,B | 19.78 | 22.83 |
| Dicamba | B | 20.18 | 23.42 |
| Dichlorprop | A | 22.53 | 25.90 |
| 2,4-D | B | 23.13 | 27.01 |
| 4,4'-Dibromooctafluorobiphenyl (IS) | A,B | 24.26 | 26.57 |
| Pentachlorophenol | A | 25.03 | 27.23 |
| Silvex | B | 25.82 | 29.08 |
| 5-Hydroxydicamba | B | 26.28 | 30.18 |
| 2,4,5-T | A | 26.57 | 30.33 |
| 2,4-DB | B | 27.95 | 31.47 |
| Dinoseb | A | 28.03 | 33.02 |
| Bentazon | B | 28.70 | 33.58 |
| Picloram | B | 29.93 | 35.90 |
| Dacthal | A | 31.02 | 34.32 |
| Acifluorfen | B | 35.62 | 40.58 |

[a] Analytes were divided into two groups during method development to avoid chromatographic overlap.

[b] Columns and chromatographic conditions are described in Sect. 6.12.

## TABLE 2. SINGLE LABORATORY RECOVERY, PRECISION DATA AND METHOD DETECTION LIMIT WITH FORTIFIED REAGENT WATER - LEVEL 1

| Analyte | Fortified Conc. µg/L | Mean[a] Recovery % | Relative Std. Dev. % | MDL µg/L |
|---|---|---|---|---|
| Acifluorfen | 0.50 | 70 | 21 | 0.25 |
| Bentazon | 2.50 | 70 | 11 | 0.63 |
| 2,4-D | 0.25 | 96 | 38 | 0.28 |
| 2,4-DB | 2.50 | 79 | 12 | 0.72 |
| Dacthal[b] | 0.25 | 96 | 16 | 0.13 |
| Dicamba | 0.75 | 109 | 11 | 0.28 |
| 3,5-Dichlorobenzoic acid | 1.25 | 126 | 24 | 1.23 |
| Dichlorprop | 0.25 | 106 | 15 | 0.13 |
| Dinoseb | 0.50 | 87 | 22 | 0.28 |
| 5-Hydroxydicamba | 0.75 | 90 | 12 | 0.25 |
| Pentachlorophenol | 0.25 | 103 | 18 | 0.16 |
| Picloram | 0.75 | 95 | 15 | 0.35 |
| 2,4,5-T | 0.25 | 116 | 18 | 0.16 |
| 2,4,5-TP | 0.25 | 98 | 9 | 0.06 |

[a] Based on the analyses of seven replicates.

[b] Measurement includes the mono- and diacid metabolites.

## TABLE 3. SINGLE LABORATORY RECOVERY AND PRECISION DATA FOR FORTIFIED REAGENT WATER - LEVEL 2

| Analyte | Fortified Conc. µg/L | Mean[a] Recovery % | Relative Std. Dev. % |
|---|---|---|---|
| Acifluorfen | 0.80 | 61 | 27 |
| Bentazon | 4.0 | 81 | 8 |
| 2,4-D | 0.40 | 96 | 38 |
| 2,4-DB | 4.0 | 90 | 13 |
| Dacthal[b] | 0.40 | 96 | 16 |
| Dicamba | 1.20 | 109 | 11 |
| 3,5-Dichlorobenzoic acid | 2.00 | 126 | 24 |
| Dichlorprop | 0.40 | 76 | 21 |
| Dinoseb | 0.80 | 87 | 22 |
| 5-Hydroxydicamba | 1.20 | 90 | 12 |
| Pentachlorophenol | 0.40 | 66 | 26 |
| Picloram | 1.20 | 68 | 21 |
| 2,4,5-T | 0.40 | 116 | 18 |
| 2,4,5-TP | 0.40 | 105 | 7 |

a Based on the analyses of six-seven replicates.

b Measurement includes the mono- and diacid metabolites.

## TABLE 4. SINGLE LABORATORY RECOVERY AND PRECISION DATA FOR FORTIFIED REAGENT WATER - LEVEL 3

| Analyte | Fortified Conc. µg/L | Mean[a] Recovery % | Relative Std. Dev. % |
|---|---|---|---|
| Acifluorfen | 2.0 | 59 | 13 |
| Bentazon | 10.0 | 68 | 8 |
| 2,4-D | 1.0 | 90 | 20 |
| 2,4-DB | 10.0 | 74 | 6 |
| Dacthal[b] | 1.0 | 60 | 10 |
| Dicamba | 3.0 | 75 | 9 |
| 3,5-Dichlorobenzoic acid | 5.0 | 62 | 18 |
| Dichlorprop | 1.0 | 97 | 17 |
| Dinoseb | 2.0 | 63 | 10 |
| 5-Hydroxydicamba | 3.0 | 77 | 8 |
| Pentachlorophenol | 1.0 | 69 | 11 |
| Picloram | 3.0 | 66 | 9 |
| 2,4,5-T | 1.0 | 64 | 15 |
| 2,4,5-TP | 1.0 | 68 | 8 |

[a] Based on the analyses of six-seven replicates.

[b] Measurement includes the mono- and diacid metabolites.

## TABLE 5. SINGLE LABORATORY RECOVERY AND PRECISION DATA FOR FORTIFIED, DECHLORINATED TAP WATER - LEVEL 1

| Analyte | Fortified Conc. µg/L | Mean[a] Recovery % | Relative Std. Dev. % |
|---|---|---|---|
| Acifluorfen | 0.50 | 117 | 21 |
| Bentazon | 2.50 | 96 | 12 |
| 2,4-D | 0.25 | 59c | 55 |
| 2,4-DB | 2.50 | 112 | 15 |
| Dacthal[b] | 0.25 | 101 | 10 |
| Dicamba | 0.75 | 91 | 14 |
| 3,5-Dichlorobenzoic acid | 1.25 | 103 | 15 |
| Dichlorprop | 0.25 | 218[d] | 37 |
| Dinoseb | 0.50 | 134 | 10 |
| 5-Hydroxydicamba | 0.75 | 90 | 14 |
| Pentachlorophenol | 0.25 | 91 | 8 |
| Picloram | 0.75 | 76 | 28 |
| 2,4,5-T | 0.25 | 118 | 16 |
| 2,4,5-TP | 0.25 | 99 | 10 |

---

[a] Based on the analyses of six-seven replicates.

[b] Measurement includes the mono- and diacid metabolites.

[c] 2,4-D background value was 0.29 µg/L.

[d] Probable interference.

## TABLE 6. SINGLE LABORATORY RECOVERY AND PRECISION DATA FOR FORTIFIED, DECHLORINATED TAP WATER - LEVEL 2

| Analyte | Fortified Conc. µg/L | Mean[a] Recovery % | Relative Std. Dev. % |
|---|---|---|---|
| Acifluorfen | 2.0 | 150 | 7 |
| Bentazon | 10.0 | 112 | 9 |
| 2,4-D | 1.0 | 90 | 16 |
| 2,4-DB | 10.0 | 111 | 10 |
| Dacthal[b] | 1.0 | 118 | 8 |
| Dicamba | 3.0 | 86 | 10 |
| 3,5-Dichlorobenzoic acid | 5.0 | 111 | 5 |
| Dichlorprop | 1.0 | 88 | 30 |
| Dinoseb | 2.0 | 121 | 6 |
| 5-Hydroxydicamba | 3.0 | 96 | 6 |
| Pentachlorophenol | 1.0 | 96 | 6 |
| Picloram | 3.0 | 132 | 12 |
| 2,4,5-T | 1.0 | 108 | 10 |
| 2,4,5-TP | 1.0 | 115 | 7 |
| 2,4-Dichlorophenylacetic acid[c] | 1.0 | 120 | 19 |

a Based on the analyses of six-seven replicates.

b Measurement includes the mono- and diacid metabolites.

c Surrogate analyte.

## TABLE 7. SINGLE LABORATORY RECOVERY AND PRECISION DATA FOR FORTIFIED, OZONATED SURFACE WATER - LEVEL 1

| Analyte | Fortified Conc. µg/L | Mean[a] Recovery % | Relative Std. Dev. % |
|---|---|---|---|
| Acifluorfen | 0.50 | 172 | 14 |
| Bentazon | 2.50 | 92 | 22 |
| 2,4-D | 0.25 | 127 | 13 |
| 2,4-DB | 2.50 | 154 | 19 |
| Dacthal[b] | 0.25 | 113 | 17 |
| Dicamba | 0.75 | 107 | 13 |
| 3,5-Dichlorobenzoic acid | 1.25 | 100 | 17 |
| Dichlorprop | 0.25 | 115 | 20 |
| Dinoseb | 0.50 | 134 | 28 |
| 5-Hydroxydicamba | 0.75 | 89 | 13 |
| Pentachlorophenol | 0.25 | 110 | 22 |
| Picloram | 0.75 | 109 | 27 |
| 2,4,5-T | 0.25 | 102 | 19 |
| 2,4,5-TP | 0.25 | 127 | 8 |
| 2,4-Dichlorophenylacetic acid[c] | 0.25 | 72 | 31 |

[a] Based on the analyses of six-seven replicates.

[b] Measurement includes the mono- and diacid metabolites.

[c] Surrogate analyte.

## TABLE 8. SINGLE LABORATORY RECOVERY AND PRECISION DATA FOR FORTIFIED, OZONATED SURFACE WATER - LEVEL 2, STABILITY STUDY DAY 1[c]

| Analyte | Fortified Conc. µg/L | Mean[a] Recovery % | Relative Std. Dev. % |
|---|---|---|---|
| Acifluorfen | 2.0 | 173 | 11 |
| Bentazon | 10.0 | 122 | 7 |
| 2,4-D | 1.0 | 126 | 10 |
| 2,4-DB | 10.0 | 130 | 7 |
| Dacthal[b] | 1.0 | 116 | 11 |
| Dicamba | 3.0 | 109 | 9 |
| 3,5-Dichlorobenzoic acid | 5.0 | 115 | 11 |
| Dichlorprop | 1.0 | 116 | 11 |
| Dinoseb | 2.0 | 116 | 9 |
| 5-Hydroxydicamba | 3.0 | 121 | 9 |
| Pentachlorophenol | 1.0 | 118 | 10 |
| Picloram | 3.0 | 182 | 14 |
| 2,4,5-T | 1.0 | 112 | 9 |
| 2,4,5-TP | 1.0 | 122 | 10 |
| 2,4-Dichlorophenylacetic acid[d] | 1.0 | 110 | 26 |

a Based on the analyses of six-seven replicates.

b Measurement includes the mono- and diacid metabolites.

c Samples preserved at pH = 2.0.

d Surrogate analyte.

## TABLE 9. SINGLE LABORATORY RECOVERY AND PRECISION DATA FOR FORTIFIED, OZONATED SURFACE WATER - LEVEL 2, STABILITY STUDY DAY 14[c]

| Analyte | Fortified Conc. µg/L | Mean[a] Recovery % | Relative Std. Dev. % |
|---|---|---|---|
| Acifluorfen | 2.0 | 151 | 18 |
| Bentazon | 10.0 | 97 | 9 |
| 2,4-D | 1.0 | 84 | 11 |
| 2,4-DB | 10.0 | 128 | 10 |
| Dacthal[b] | 1.0 | 116 | 7 |
| Dicamba | 3.0 | 103 | 9 |
| 3,5-Dichlorobenzoic acid | 5.0 | 81 | 12 |
| Dichlorprop | 1.0 | 107 | 11 |
| Dinoseb | 2.0 | 118 | 7 |
| 5-Hydroxydicamba | 3.0 | 20 | 14 |
| Pentachlorophenol | 1.0 | 94 | 7 |
| Picloram | 3.0 | 110 | 32 |
| 2,4,5-T | 1.0 | 113 | 8 |
| 2,4,5-TP | 1.0 | 113 | 11 |
| 2,4-Dichlorophenylacetic acid[d] | 1.0 | 87 | 6 |

[a] Based on the analyses of six-seven replicates.

[b] Measurement includes the mono- and diacid metabolites.

[c] Samples preserved at pH = 2.0.

[d] Surrogate analyte.

## TABLE 10. SINGLE LABORATORY RECOVERY AND PRECISION DATA FOR FORTIFIED, HIGH HUMIC CONTENT SURFACE WATER

| Analyte | Fortified Conc. µg/L | Mean[a] Recovery % | Relative Std. Dev. % |
|---|---|---|---|
| Acifluorfen | 2.0 | 120 | 13 |
| Bentazon | 10.0 | 87 | 11 |
| 2,4-D | 1.0 | 59 | 7 |
| 2,4-DB | 10.0 | 80 | 14 |
| Dacthal[b] | 1.0 | 100 | 6 |
| Dicamba | 3.0 | 76 | 9 |
| 3,5-Dichlorobenzoic acid | 5.0 | 87 | 4 |
| Dichlorprop | 1.0 | 110 | 22 |
| Dinoseb | 2.0 | 97 | 6 |
| 5-Hydroxydicamba | 3.0 | 82 | 9 |
| Pentachlorophenol | 1.0 | 70 | 5 |
| Picloram | 3.0 | 124 | 9 |
| 2,4,5-T | 1.0 | 101 | 4 |
| 2,4,5-TP | 1.0 | 80 | 6 |

[a] Based on the analyses of six-seven replicates.

[b] Measurement includes the mono- and diacid metabolites.

## TABLE 11. LABORATORY PERFORMANCE CHECK SOLUTION

| Test | Analyte | Conc, µg/mL | Requirements |
|---|---|---|---|
| Sensitivity | Dinoseb | 0.004 | Detection of analyte; S/N > 3 |
| Chromatographic performance | 4-Nitrophenol | 1.6 | 0.70 <PGF<1.05[b] |
| Column performance | 3,5-Dichlorobenzoic acid<br>4-Nitrophenol | 0.6<br>1.6 | Resolution >0.40[b] |

[a] PGF = peak Gaussian factor. Calculated using the equation:

$$PGF = \frac{1.83 \times W(1/2)}{W(1/10)}$$

where W(1/2) is the peak width at half height and W(1/10) is the peak width at tenth height.

[b] Resolution between the two peaks as defined by the equation:

$$R = \frac{t}{W}$$

where t is the difference in elution times between the two peaks and W is the average peak width, at the baseline, of the two peaks.

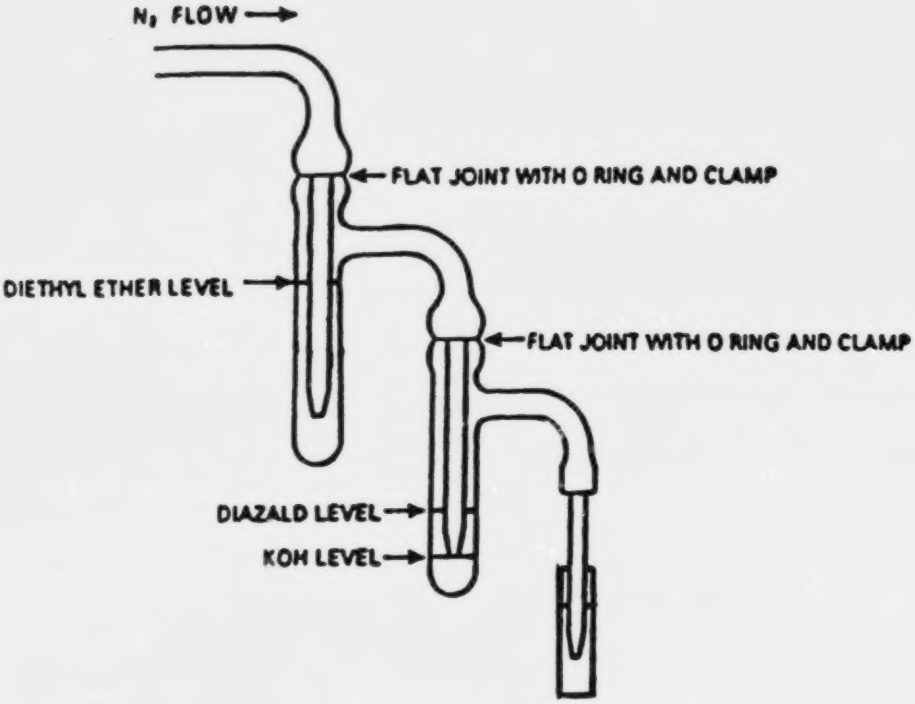

$N_2$ FLOW
FLAT JOINT WITH O RING AND CLAMP
DIETHYL ETHER LEVEL
FLAT JOINT WITH O RING AND CLAMP
DIAZALD LEVEL
KOH LEVEL

FIGURE 1. DIAZOMETHANE GENERATOR

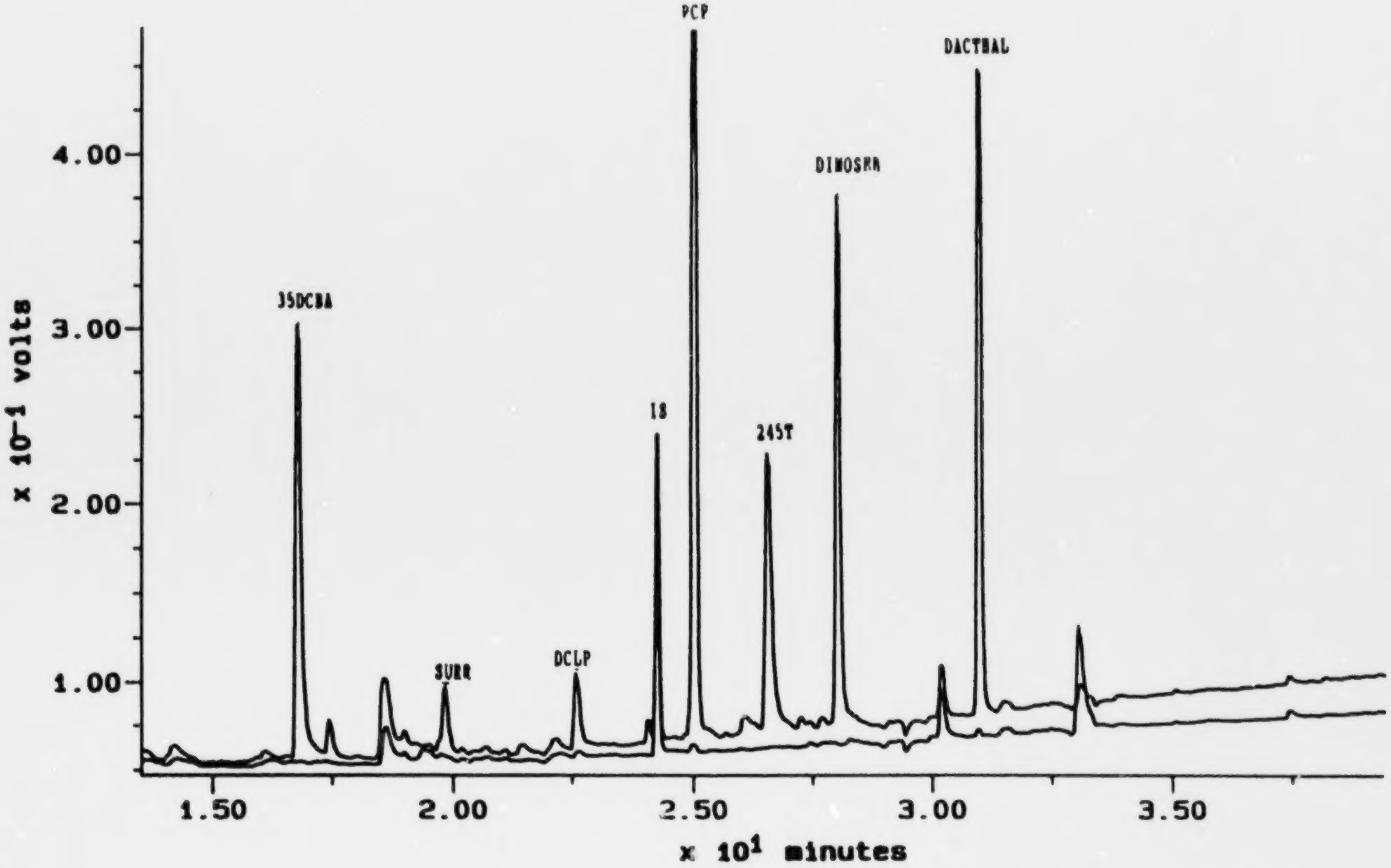


Figure 2A. Chromatogram of Group A Analytes Extracted From Ozonated Surface Water (bottom chromatogram is the laboratory reagent blank)

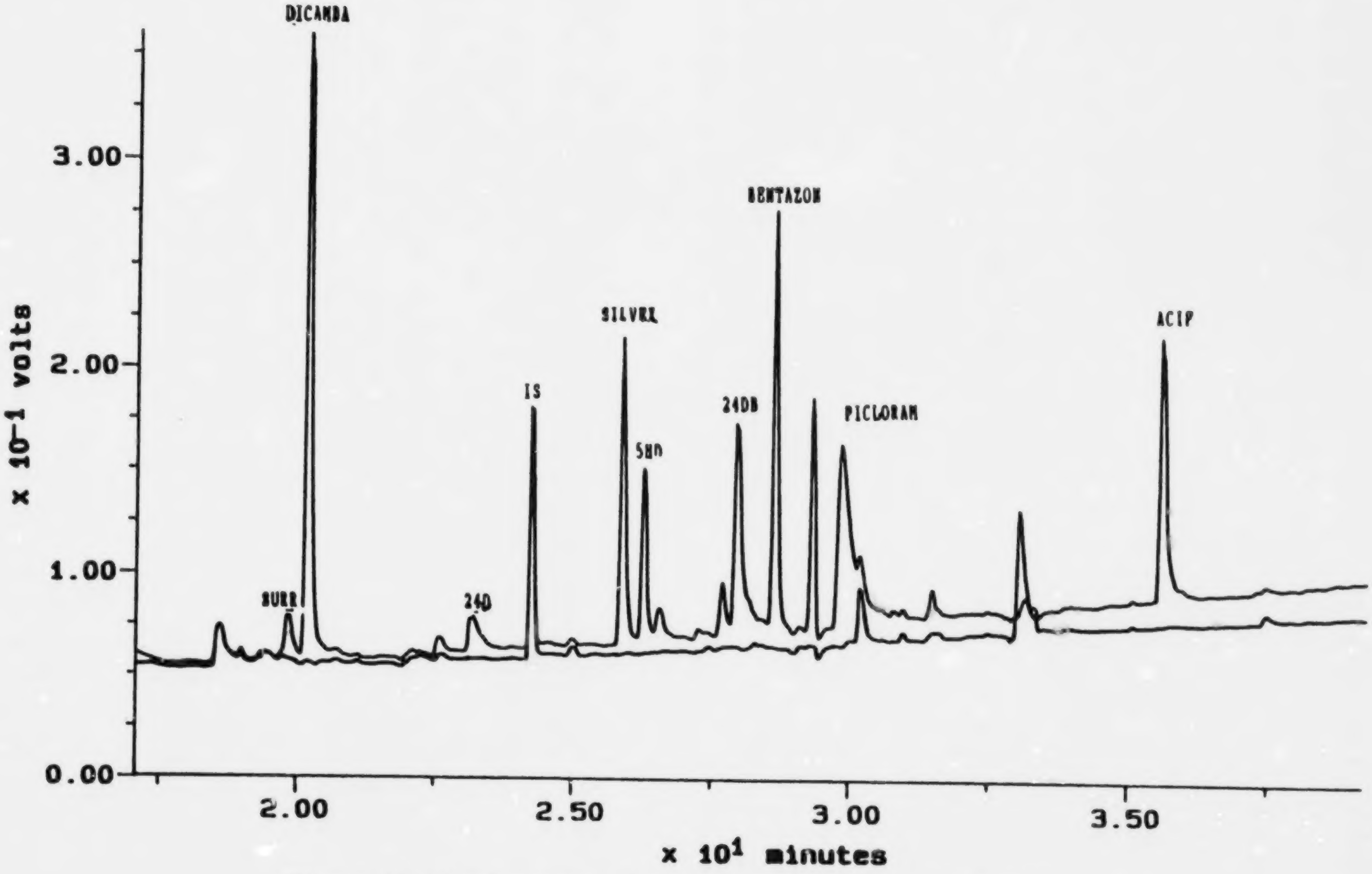


Figure 2B. Chromatogram of Group B Analytes Extracted From Ozonated Surface Water (bottom chromatogram is the laboratory reagent blank)

# METHOD 548.1 DETERMINATION OF ENDOTHALL IN DRINKING WATER BY ION-EXCHANGE EXTRACTION, ACIDIC METHANOL METHYLATION AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY

Revision 1.0

August 1992

Jimmie W. Hodgeson

Jeffrey Collins (Technology Applications, Incorporated)

W. J. Bashe (Technology Applications, Incorporated)

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

# METHOD 548.1

## DETERMINATION OF ENDOTHALL IN DRINKING WATER BY ION EXCHANGE EXTRACTION, ACIDIC METHANOL METHYLATION AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY

## 1. SCOPE AND APPLICATION

1.1 This method is for the identification and simultaneous measurement of endothall in drinking water sources and finished drinking water. The following analyte can be determined by this method:

| Analyte | Chemical Abstract Services Registry Number |
|---|---|
| Endothall | 145-73-3 |

1.2 This is a gas chromatographic/mass spectrometric (GC/MS) method. However, a flame ionization detector (FID) may be utilized for the determination, but must be supported by an additional analysis using a confirmatory gas chromatographic column.

1.3 The method detection limit (1) (MDL, defined in Sect. 13) for endothall is listed in Table 1 for both GC/MS and FID. The MDL may differ from the listed value depending upon the nature of interferences in the sample matrix. In particular, water sources containing high levels of dissolved calcium, magnesium and sulfate may require sample dilution before extraction to obtain adequate endothall recovery. Guidelines (Sect. 4.2 and Sect. 11.2.1) are provided on levels of these ions above which dilution is recommended, as well as appropriate dilution factors.

1.4 In this ion exchange liquid-solid extraction procedure, endothall may be esterified directly in the elution solvent, acidic methanol.

1.5 The method performance data provided in this method were obtained using both a GC/MS system and a gas chromatograph with a flame ionization detector (FID). Modern GC/MS instruments have sensitivities at least equivalent to the FID. If the analyst has access to a GC/MS system meeting the specifications described in Sect. 6.10, it should be as the primary means of identification and measurement.

## 2. SUMMARY OF METHOD

2.1 Liquid-solid extraction (LSE) cartridges containing an intermediate strength, primarily tertiary amine anion exchanger are mounted on a vacuum manifold and conditioned with appropriate solvents. LSE disks may be used instead of cartridges of all quality control criteria specified in Sect. 9 are met. A 100-mL sample is extracted and the analyte is eluted with 8-mL of acidic methanol. After addition of a small volume of methylene chloride as a co-solvent, the dimethyl ester of endothall is formed within 30 min with modest heating (50°C). After addition of salted reagent water, the ester is partitioned into 8-10 mL of methylene chloride. The extract volume is reduced to 1 mL with nitrogen purge for a concentration factor of 100. The extract is analyzed by GC/MS or GC/FID with a megabore capillary column.

## 3. DEFINITIONS

3.1 INTERNAL STANDARD (IS) -- A pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same sample or solution. The internal standard must be an analyte that is not a sample component.

3.2 SURROGATE ANALYTE (SA) -- A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction or other processing and is measured with the same procedures used to measure other sample components. The purpose of the SA is to monitor method performance with each sample.

3.3 LABORATORY DUPLICATES (LD1 and LD2) -- Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 FIELD DUPLICATES (FD1 and FD2) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.5 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 FIELD REAGENT BLANK (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.7 INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

3.8 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.9 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.10 STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.11 PRIMARY DILUTION STANDARD SOLUTION (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.12 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.13 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes in known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

## 4. INTERFERENCES

4.1 Method interference may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the analytical conditions by analyzing laboratory reagent blanks as described in Sect. 9.2.

4.1.1 Glassware must be scrupulously cleaned (2) as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and distilled water. It should then be drained dry, and heated in a laboratory oven at 400°C for several hours before use. Solvent rinses with methanol may be substituted for the oven heating. After drying and cooling, glassware should be stored in a clean environment to prevent any accumulation of dust or other contaminants.

4.1.2 The use of high purity reagents and solvents is absolutely necessary to minimize interference problems. Purification of solvents by distillation in all-glass systems immediately prior to use may be necessary.

4.2 The major potential interferences in this ion-exchange procedure are other naturally occurring ions in water sources, namely, dissolved calcium, magnesium and sulfate. These are the only ions thus far demonstrated to be interferences when present at concentrations possibly occurring in drinking water sources. For example, the sources identified in Tables 3 and 4 contained elevated concentrations of these ions and reduced recoveries were observed. Sulfate is an effective counter ion, and displaces endothall from the column when present at high concentrations. On the other hand, both calcium and magnesium complex the endothall anion, which then is no longer available in ionic form for ion-exchange extraction. Table 4 illustrates that sample dilution or the addition of ethylenediamine tetraacetic acid for complexing the cations, or a combination of the two, may be used. Figure 1 illustrates quantitatively the separate effects of these ions on recovery.

4.3 The extent of interferences that may be encountered using this method has not been fully assessed. Although the GC conditions described allow for a unique resolution of endothall, other matrix components may interfere. Matrix interferences may be caused by contaminants that are coextracted from the sample. Matrix interferences will vary considerably from source to source, depending on the nature of the matrix being sampled. A distinct advantage of this method is that the anion exchange cartridge provides an effective clean-up mechanism for many potential organic matrix interferences. Many neutral and basic organics retained by the column are removed by the methanol wash step of Sect. 11.2.3. The most probable matrix interferences are other organic acids or phenols retained by the column. For the cartridge to effectively serve for both sample clean-up and analyte extraction, it is critical that the conditioning steps described in Sect. 11.2.1 be followed exactly.

## 5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be minimized. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemical specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additionally references to laboratory safety are available (3-5).

## 6. EQUIPMENT AND SUPPLIES

6.1 SAMPLING EQUIPMENT (for discrete or composite sampling). Amber glass bottles (250 mL or larger) fitted with screw caps lined with Teflon. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with methanol, and dried before use to minimize contamination.

6.2 SEPARATOR FUNNELS -- 125 mL, with Teflon stopcocks, ground glass or Teflon stoppers.

6.3 SCREW CAP -- 125 x 13 mm, culture tubes. Screw caps should have Teflon liners.

6.4 Graduated 15 mL centrifuge tubes with #13 ground glass stoppers

6.5 PASTEUR PIPETS -- Glass, disposable 5-3/4" length

6.6 BALANCE -- Analytical, capable of weighing to .0001 g.

6.7 Six or twelve position analytical concentrator (Organomation, N-EVAP model # 111/6917 or equivalent).

6.8 pH METER

6.9 GAS CHROMATOGRAPH -- Analytical system complete with GC suitable for flame ionization detection, split/splitless capillary injection temperature programming, and all required accessories including syringes, analytical columns, gases and strip chart recorder. A data system is recommended for measuring peak areas. An auto injector is recommended for improved precision of analysis.

6.10 GAS CHROMATOGRAPH/MASS SPECTROMETER/DATA SYSTEM (GC/MS/DS) --

6.10.1 The GC must be capable of temperature programming and be equipped for split/splitless or on-column capillary injection. The injection tube liner should be quartz and about

3 mm in diameter. The injection system must not allow the analytes to contact hot stainless steel or other metal surfaces that promote decomposition.

6.10.2 The GC/MS interface should allow the capillary column or transfer line exit to be placed within a few mm of the ion source. Other interfaces, for example, the open split interface, are acceptable as long as the system has adequate sensitivity (See Sect. 10 for calibration requirements).

6.10.3 The mass spectrometer must be capable of electron ionization at a nominal electron energy of 70 eV and of scanning from 45 to 450 amu with a complete scan cycle time (including scan overhead) of 1.5 sec or less. (Scan cycle time = Total MS data acquisition time in sec divided by total number of scans in the chromatogram). The spectrometer must produce a mass spectrum that meets all criteria in Table 5 when 5 to 10 ng of DFTPP is introduced into the GC. An average spectrum across the DFTPP GC peak may be used to test instrument performance.

6.10.4 An interfaced data system is required to acquire, store, reduce, and output mass spectral data. The computer software must have the capability of processing stored data by recognizing a GC peak within any given retention time window, comparing the mass spectra from the GC peak with spectral data in a user-created data base, and generating a list of tentatively identified compounds with their retention times and scan numbers. The software must also allow integration of the ion abundance of any specific ion between specified time or scan number limits, calculation of response factors as defined in Sect. 10.3.6 (or construction of a second or third order regression calibration curve), calculation of response factor statistics (mean and standard deviation), and calculation of concentrations of analytes as described in Sect. 12.

6.11 GC COLUMNS

6.11.1 GC/MS -- DB5, 30 m x 0.25 mm, 0.25 µm film thickness

6.11.2 FID Primary -- RTX Volatiles, 30 m x 0.53 mm. ID, 2.0 µm film thickness, Restek Catalog No. 10902.

6.11.3 FID Confirmation -- DB5, 30 m x 0.32 mm ID, 0.25 µm film thickness

6.12 LIQUID-SOLID EXTRACTION VACUUM SYSTEM -- May be used.

6.13 8 mL LIQUID-SOLID EXTRACTION CARTRIDGES WITH FRITS -- Also available from a number of commercial suppliers. Appropriate liquid-solid extraction disks may also be used in this method if equivalent or better quality assurance data can be demonstrated (See Sect. 9).

6.14 LIQUID-SOLID EXTRACTION 70 mL RESERVOIRS AND ADAPTERS -- Baxter Catalog # 9442 (adapter catalog # 9430) or equivalent.

## 7. **REAGENTS AND CONSUMABLE MATERIALS**

7.1 REAGENT WATER -- Reagent water is defined as water in which an interference is not observed at the endothall method detection.

7.1.1 A Millipore Super-Q Water System or its equivalent may be used to generate deionized reagent water. Distilled water that has been charcoal filtered may also be suitable.

7.2 METHANOL -- Pesticide quality.

7.3 METHYLENE CHLORIDE -- Pesticide quality or equivalent.

7.4 SODIUM SULFATE-ACS GRANULAR -- Heat in a shallow tray for 4 hrs at 400°C to remove phthlates and other interfering organic substances or extract with methylene chloride in a Soxhlet apparatus for 48 hrs.

7.5 10% SULFURIC ACID IN METHANOL -- Using extreme caution, slowly dissolve reagent grade sulfuric (10% v/v) acid in methanol.

7.6 SODIUM HYDROXIDE (NAOH) 1 N -- Dissolve 4 g ACS grade in reagent water and dilute up to 100 mL in a 100 mL volumetric flask.

7.7 10% SODIUM SULFATE IN REAGENT WATER -- Dissolve 100 g sodium sulfate in reagent water and dilute to volume in a 1-L volumetric flask.

7.8 BIOREX 5 ANION EXCHANGE RESIN -- BioRad Laboratories Catalog No. 140-7841.

7.9 DISODIUM ETHYLENEDIAMINE TETRAACETATE (EDTA) -- Certified ACS Fisher or equivalent.

7.10 ENDOTHALL, MONOHYDRATE -- Available as neat material from Ultra Scientific, North Kingston, RI or as a concentrated solution from NSI Environmental Solutions, Research Triangle Park, NC.

7.11 ACENAPTHENE-d10 -- Available from MSD Isotopes or Cambridge Chemicals.

7.12 STOCK STANDARD SOLUTIONS

7.12.1 Endothall -- 50 µg/mL in methanol

7.12.2 Acenaphthene-d10 -- 500 µg/mL in methanol. Dissolve 25 mg (approximately 32.2 µL) Acenapthnene-d10 in 50 mL methanol. Prepare a working standard at 10 µg/mL by a 1:50 dilution of the stock standard.

7.12.3 Decafluorotriphenylphosphine (DFTPP) -- 5 µg/mL.

7.12.4 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

## 8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Grab samples must be collected in glass containers. Conventional sampling practices should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers. Automatic sampling equipment must be as free as possible of plastic tubing and other potential sources of contamination.

### 8.2 SAMPLE PRESERVATION

8.2.1 If residual chlorine is present, add 80 mg of sodium thiosulfate per liter of sample to the sample bottle prior to collecting the sample.

8.2.2 After adding the sample to the bottle containing the sodium thiosulfate, seal the bottle and shake vigorously for 1 min.

8.2.3 The samples must be iced or refrigerated at 4°C from the time of collection until extraction and analysis. Endothall is not known to be light sensitive, but excessive exposure to light and heat should be avoided.

8.2.4 A graphical representation of the results of a 14-day holding stability study on endothall in three different water matrices is presented in Figure 2. These matrices were a dechlorinated tap water sample, a filtered river water sample containing considerable biological activity and the same river water biologically preserved at pH 2. These data indicate that the samples may be held for 7 days before extraction under the conditions of Sect. 8.2.3. Endothall appears to be biologically stable over 7 days. However, the chemical and biological stability of endothall may be matrix dependent. The analyst may verify analyte stability in the matrix of interest by conducting appropriate holding studies. Samples with unusually high biological activity should be acidified to pH 1.5 to 2.0 with 1:1 $HCl:H_2O$.

8.3 EXTRACT STORAGE -- Sample extracts should be stored in the dark at 4°C or less. A maximum extract holding time of 14 days is recommended.

## 9. QUALITY CONTROL

9.1 Each laboratory that uses this method is required to operate a formal quality control (QC) program. The minimum QC requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified blanks, laboratory fortified matrix samples and QC check standards.

9.2 LABORATORY REAGENT BLANKS -- Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is analyzed or reagents are changed, a laboratory reagent blank must be analyzed. For this method, the blank matrix is filtered reagent water. If within the retention time window of endothall, the reagent blank produces a peak which prevents the measurement of endothall, determine the source of contamination and eliminate the interference before processing samples.

9.3 INITIAL DEMONSTRATION OF CAPABILITY

9.3.1 Select a representative fortified concentration for endothall. Prepare a methanol solution containing endothall at 1000 times the selected concentration. The concentrate must be prepared independently from the standards used to prepare the calibration curve (Sect. 10.2). With a syringe, add 100 µL of the concentrate to each of four to seven 100-mL aliquots of reagent water and analyze each aliquot according to procedures in Sect. 11.

9.3.2 Calculate the mean percent recovery (R), the relative standard deviation of the recovery (RSD in Table 2), and the MDL (1). The mean recovery must fall in the range of R ± 20% using the values for R (Recovery) for reagent water (Table 2). The standard deviation should be less than 30%. If these acceptance criteria are met, performance is acceptable and sample analysis may begin. If either of these criteria fails, initial demonstration of capability should be repeated until satisfactory performance has been demonstrated.

9.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples by a new, unfamiliar method prior to demonstrating a basic level of skill at performing the technique. As laboratory personnel gain experience with this method the quality of the data should improve beyond the requirements stated in Sect. 9.3.2.

9.4 The analyst is permitted to modify GC columns or GC conditions to improve separations or lower analytical costs. Each time such method modifications are made, the analyst must repeat the procedures in Sect. 9.3.

9.5 ASSESSING THE INTERNAL STANDARD -- In using the IS calibration procedure, the analyst is expected to monitor the IS response (peak area) of all samples during each analysis day. The IS response for any sample chromatogram should not deviate from the most recent calibration check standard IS response by more than 30%.

9.5.1 If a deviation of greater than 30% is encountered for a sample, reinject the extract.

9.5.1.1 If acceptable IS response is achieved for the reinjected extract, then report the results for that sample.

9.5.1.2 If a deviation of greater than 30% is obtained for the reinjected extract, analysis of the sample should be repeated beginning with Sect. 11, provided the sample is still available. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.

9.5.2 If consecutive samples fail the IS response acceptance criterion, immediately analyze a medium calibration check standard.

9.5.2.1 If the check standard provides a response factor (RF) within 20% of the predicted value, then follow procedures itemized in Sect. 9.5.1 for each sample failing the IS response criterion.

9.5.2.2 If the check standard provides a response factor (RF) which deviates more than 20% from the predicted value, then the analyst must recalibrate, as specified in Sect. 10.2.

9.6 ASSESSING LABORATORY PERFORMANCE

9.6.1 The laboratory must analyze at least one laboratory fortified blank (LFB) per sample set (all samples extracted within a 24-hr period). The fortifying concentration in the LFB should be 10 to 20 times the MDL. Calculate accuracy as percent recovery ($R_i$). If the recovery falls outside the control limits (See Sect. 9.6.2), the system is judged out of control, and the source of the problem must be identified and resolved before continuing analyses.

9.6.2 Until sufficient LFB data become available, usually a minimum of results from 20 to 30 analyses, the laboratory should assess its performance against the control limits described in Sect. 9.3.2. When sufficient laboratory performance data become available, develop control limits from the mean percent recovery (R) and standard deviation (S) of the percent recovery. These data are used to establish upper and lower control limits as follows:

Upper Control Limit = R + 3S
Lower Control Limit = R - 3S

After each group of five to ten new recovery measurements, control limits should be recalculated using only the most recent 20 to 30 data points.

9.6.3 Each laboratory should periodically determine and document its detection limit capabilities for endothall.

9.6.4 Each quarter the laboratory should analyze quality control samples (if available). If criteria provided with the QCS are not met, corrective action should be taken and documented.

## 9.7 ASSESSING ANALYTE RECOVERY

9.7.1 The laboratory must add a known fortified concentration to a minimum of 10% of samples or one fortified matrix sample per set, whichever is greater. The fortified concentration should not be less than the background concentration of the sample selected for fortification. The fortified concentration should be the same as that used for the LFB (Sect. 9.6). Over time, samples from all routine sample sources should be fortified.

9.7.2 Calculate the percent recovery for endothall, corrected for background concentrations measured in the unfortified sample, and compare these values to the control limits established in Sect. 9.6.2 for the analyses of LFBs.

9.7.3 If the recovery falls outside the designated range and the laboratory performance for that sample set is shown to be in control (Sect. 9.6), the recovery problem encountered with the fortified sample is judged to be matrix related, not system related. The result in the unfortified sample must be labelled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

## 10. CALIBRATION AND STANDARDIZATION

### 10.1 PREPARATION OF CALIBRATION STANDARDS

10.1.1 Calibration standards as dimethyl esters are prepared by addition of aliquots of the endothall stock standard (Sect. 7.12.1) to the esterification reaction mixture, consisting of 8 mL of 10% $H_2SO_4$/methanol and 6 mL of methylene chloride in the screw cap culture tubes (Sect. 6.3). The standards are then esterified and partitioned into the organic phase according to Sect. 11.4. Prepare endothall acid standards equivalent to aqueous standards at 100, 50, 25 and 5 µg/L by addition of the following aliquots of the stock standard solution (Sect. 7.12) to the esterification reaction mixture - 200 µL, 100 µL, 50 µL and 10 µL. By way of illustration, 200 µL of the 50 µg/mL stock contains 10 µg of endothall. When dissolved in 100 mL of water, the aqueous concentration is 100 µg/L.

10.1.2 Process each standard as described in Sect. 11.4.1 and Sect. 11.4.2. The internal standard is added as described in Sect. 11.4.3. Triplicate samples should be prepared at each concentration level.

10.2 Demonstration and documentation of acceptable initial calibration are required before any samples are analyzed and intermittently throughout sample analyses as dictated by results of continuing calibration checks. After initial calibration is successful, a continuing calibration check is required at the beginning of each 8-hr period during which analyses are performed. Additional periodic calibration checks are good laboratory practice.

### 10.3 INITIAL CALIBRATION

10.3.1 Calibrate the mass spectrometer with calibration compounds and procedures prescribed by the manufacturer with any modifications necessary to meet the requirements in Sect. 10.3.2.

10.3.2 Inject into the GC a 1-or 2-µL aliquot of the 5 ng/µL DFTPP solution and acquire a mass spectrum that includes data for m/z = 45-450. Use GC conditions that produce a narrow (at least five scans per peak) symmetrical peak. If the spectrum does not meet all criteria (Table 5), the MS must be retuned to meet all criteria before proceeding with calibration. An average spectrum across the GC peak may be used to evaluate the performance of the system.

10.3.3 Inject a 1-µL aliquot of a medium concentration calibration solution, for example 50 µg/L, and acquire and store data from m/z 45-450 with a total cycle time (including scan overhead time) of 1.5 sec or less. Cycle time should be adjusted to measure at least five or more spectra during the elution of the GC peak. Figure 3 illustrates a total ion chromatogram and mass spectrum of endothall and the internal standard, acenaphthene-d10, using the prescribed conditions.

10.3.4 If all performance criteria are met, inject a 1-µL aliquot of each of the other calibration solutions using the same GC/MS conditions.

10.3.5 Calculate a response factor (RF) for endothall for each calibration solution by use of the internal standard response as expressed below. This calculation is supported in acceptable GC/MS data system software (Sect. 6.10.4), and many other software programs. The RF is a unitless number, but units used to express quantities of analyte and internal standard must be equivalent.

$$RF = \frac{(A_x)(Q_{is})}{(A_{is})(Q_x)}$$

where:
$A_x$ = integrated abundance of the quantitation ion of the analyte (m/z 183).
$A_{is}$ = integrated abundance of the quantitation ion internal standard (m/z 164).
$Q_x$ = quantity of analyte injected in ng or concentration units.
$Q_{is}$ = quantity of internal standard injected in ng or concentration units.

10.3.5.1 Calculate the mean RF from the analyses of the calibration solutions. Calculate the standard deviation (SD) and the relative standard deviation (RSD) from each mean: RSD = 100 (SD/M). If the RSD of any analyte or surrogate mean RF exceeds 30%, either analyze additional aliquots of appropriate calibration solutions to obtain an acceptable RSD of RFs over the entire concentration range or take action to improve GC/MS performance. See Sect. 10.4.5 for possible remedial actions.

10.3.6 As an alternative to calculating mean response factors and applying the RSD test, use the GC/MS data system software or other available software to generate a linear or second order regression calibration curve.

10.4 Continuing calibration check. Verify the MS tune and initial calibration at the beginning of each 8-hr work shift during which analyses are performed using the following procedure.

10.4.1 Inject a 1-$\mu$L aliquot of the 5 ng/$\mu$L DFTPP solution and acquire a mass spectrum that includes data for m/z 45-450. If the spectrum does not meet all criteria (Table 5), the MS must be retuned to meet all criteria before proceeding with the continuing calibration check.

10.4.2 Inject a 1-$\mu$L aliquot of a medium concentration calibration solution and analyze with the same conditions used during the initial calibration.

10.4.3 Determine that the absolute area of the quantitation ion of the internal standard has not decreased by more than 30% from the area measured in the most recent continuing calibration check, or by more than 50% from the area measured during initial calibration. If the area has decreased by more than these amounts, adjustments must be made to restore system sensitivity. These adjustments may require cleaning of the MS ion source, or other maintenance as indicated in Sect. 10.4.5, and recalibration. Control charts are useful aids in documenting system sensitivity changes.

10.4.4 Calculate the RF for endothall from the data measured in the continuing calibration check. The RF must be within 30% of the mean value measured in the initial calibration. Alternatively, if a linear or second order regression is used, the concentration measured using the calibration curve must be within 30% of the true value of the concentration in the medium calibration solution. If these conditions do not exist, remedial action must be taken which may require repeating the initial calibration.

10.4.5 Some possible remedial actions: major maintenance such as cleaning an ion source, cleaning quadrupole rods, etc. require returning to the initial calibration step.

10.4.5.1 Check and adjust GC and/or MS operating conditions; check the MS resolution, and calibrate the mass scale.

10.4.5.2 Clean or replace the splitless injection liner; silanize a new injection liner.

10.4.5.3 Flush the GC column with solvent according to manufacturer's instructions.

10.4.5.4 Break off a short portion (about 1 meter) of the column from the end near the injector; or replace GC column. This action will cause a slight change in retention times.

10.4.5.5 Prepare fresh CAL solutions, and repeat the initial calibration step.

10.4.5.6 Clean the MS ion source and rods (if a quadrupole).

10.4.5.7 Replace any components that allow analytes to come into contact with hot metal surfaces.

10.4.5.8 Replace the MS electron multiplier or any other faulty components.

## 11. **PROCEDURE**

### 11.1 PREPARATION OF ANION EXCHANGE CARTRIDGES

11.1.1 Prepare a 50% (v/v) slurry of Bio-Rex 5 resin and reagent water.

11.1.2 Attach the required number of 8-mL extraction cartridges (Sect. 6.13) to the vacuum manifold (Sect. 6.12), and insert bottom fritted disks into each cartridge.

11.1.3 Fill the cartridges completely with Bio-Rex 5 slurry. Draw off excess water with vacuum. The final wet resin bed height should be 3.5 ± 0.1 cm. Adjust the height by adding more slurry and repeating procedure, or add more reagent water to reservoir and remove excess resin slurry.

11.1.4 After the bed heights are adjusted to 3.5 cm and with excess water removed under vacuum, insert a fritted disk on top of the resin bed. The fritted disk should press firmly into the resin and be horizontal to the reservoir to prevent sample channeling around the disk. Fill the cartridges with reagent water and draw half of the water into the resin. Maintain the resin cartridges in this condition until ready for use.

NOTE: The use of liquid-solid extraction disks instead of cartridges is permissible as long as all the quality control criteria specified in Sect. 9 of this method are met.

### 11.2 SAMPLE PREPARATION

11.2.1 As discussed above (Sect. 1.3 and Sect. 4.2), reduced recoveries will be observed if the sample contains elevated levels of CaII, MgII or sulfate. If facilities are available, measure the concentrations of these ions. Figure 1 graphically presents analyte recovery versus individual ion concentration. Reduced recoveries may be anticipated when the combined CaII + MgII exceeds approximately 100 mg/L or sulfate exceeds approximately 250 mg/L. If measurement of ion concentration is not feasible, determine the actual recovery for a laboratory fortified sample matrix as described in Sect. 9.7. In the event of anticipated or measured low recoveries, treat the sample as described in Sect. 11.2.2.

11.2.2 For samples containing moderately high levels of these ions, add 186 mg of EDTA (Sect. 7.9) per 100-mL sample (0.005 M). The treated ground water characterized in Table 3 is an example of a matrix successfully treated this way. For samples containing very high levels of sulfate, sample dilution may be required in addition to the EDTA. The western surface water characterized in Table 3 (ca. 2000 mg/L sulfate) was successfully analyzed after dilution by a factor of 10 and the addition of 75 mg EDTA per 100 mL of the diluted sample (0.002 M). Samples containing intermediate levels of sulfate can be analyzed with smaller dilution factors. Guidelines on dilution factors and EDTA addition are given below.

| SULFATE, mg/L | DILUTION FACTOR | ADDED EDTA, mg/100 mL |
|---|---|---|
| < 250 | 1:1 | 186 |
| 250 - 500 | 1:2 | 125 |
| 500 - 1250 | 1:5 | 75 |
| > 1250 | 1:10 | 75 |

NOTE: Dilution should not be employed if adequate recovery is attained by the addition of EDTA alone.

11.2.3 The addition of EDTA results in a large reagent peak near the end of the temperature program. Therefore, complete the entire program described in Table 1.

11.2.4 If the ionic nature of the samples being processed is completely unknown, the analyst as an option may routinely dilute all samples by a factor of 10 and add EDTA as above. However, the analyst should be able to demonstrate reagent water MDLs of 2 µg/L or lower. In this event the MDL will be 20 µg/L or less for the diluted sample, still a factor of 5 below the regulated maximum contaminant level.

## 11.3 SAMPLE EXTRACTION

11.3.1 Attach the 70-mL reservoir to the resin cartridge with the adapter (Sect. 6.14).

11.3.2 Condition the resin cartridge by drawing the following reagents through the cartridge in the following order:

1. 10 mL methanol
2. 10 mL reagent water
3. 10 mL 10% $H_2SO_4$ in methanol
4. 10 mL reagent water
5. 20 mL 1 N NaOH
6. 20 mL reagent water

Do not allow the cartridge to become dry between steps. Draw each reagent through the cartridge at a rate of 10 mL/min. Leave a 1-cm layer of reagent water over the resin bed.

11.3.3 Fill the 70-mL reservoir with 60 mL of the sample. Adjust sample flow rate to 3 mL/min. Add the balance of sample when needed to prevent the reservoir from going dry.

11.3.4 After the sample passes through the cartridge, remove the 70-mL reservoir and the adapter. Draw 10 mL of methanol through the resin cartridge. Make sure that any visible water inside the cartridge dissolves in methanol. Next draw room air through the cartridge for 5 min under a vacuum of 10-20 in. Hg. Position the culture tube (Sect. 6.3) inside the manifold to collect the eluent.

11.3.5 Elute the cartridge with 8 mL of 10% $H_2SO_4$ in methanol, followed by 6 mL of methylene chloride under vacuum over a 1 min period.

## 11.4 SAMPLE DERIVATIZATION, PARTITION AND ANALYSIS

11.4.1 Cap the culture tube and hold at 50°C for 1 hr in a heating block or water bath. Remove from heat and allow the tube to cool for 10 min.

11.4.2 Pour the contents of the culture tube into a 125-mL separatory funnel. Rinse the tube with two x 0.5 mL aliquots of methylene chloride and add the rinsings to the separatory funnel. Add 20 mL of 10% sodium sulfate in reagent water to the separatory funnel. Shake the funnel three times vigorously, venting with the stopcock, and then shake vigorously for an additional 15 sec. After the phases have separated, drain the lower organic layer into a 15-mL graduated centrifuge tube (Sect. 6.4). Repeat the extraction procedure above with two additional 2-mL aliquots of methylene chloride, adding the organic phase to the centrifuge tube each time.

11.4.3 Fortify the extract with 250 µL of the internal standard working solution (Sect. 7.12.2) and concentrate to a final volume of 1.0 mL, using the N-EVAP (Sect. 6.7) and dry nitrogen.

11.4.4 Inject 2 µL of the concentrated extract (Sect. 11.4.3) and analyze by GC/MS using the conditions described in Table 1. This table includes the retention time and MDL that were obtained under these conditions. A sample total ion chromatogram of endothall and d-10 acenaphthene illustrating retention times, and the mass spectrum of the dimethylated endothall are shown in Figure 3. Other columns, chromatographic conditions, or detectors may be used if the requirements of Sect. 9.3 are met.

11.4.5 If the peak area exceeds the linear range of the calibration curve, a smaller sample volume should be used.

### 11.5 IDENTIFICATION OF THE ANALYTE

11.5.1 Identify endothall by comparison of its mass spectrum (after background subtraction) to a reference spectrum in a user created spectral library. The GC retention time of the sample component should be within 10 sec of the retention time of endothall in the latest calibration standard. If a FID is used, identifications should be confirmed by retention time comparisons on the second GC column (Table 1).

11.5.2 In general, all ions present above 10% relative abundance in the mass spectrum of the standard should be present in the mass spectrum of the sample component and should agree within absolute 20%. For example, if an ion has a relative abundance of 30% in the standard spectrum, its abundance in the sample spectrum should be in the range of 10-50%. However, the experience of the analyst should weigh heavily in the interpretation of spectra and chromatograms.

11.5.3 Identification requires expert judgement when sample components are not resolved chromatographically, that is, when GC peaks from interferences are present. When endothall coelutes with an interference, indicated by a broad peak or a shoulder on the peak, the identification criteria can usually be met, but the endothall spectrum will contain extraneous ions contributed by the coeluting interfering compound.

## 12. **DATA ANALYSIS AND CALCULATIONS**

12.1 When using GC/MS, complete chromatographic resolution is not necessary for accurate and precise measurements of analyte concentrations if unique ions with adequate intensities are available for quantitation. However, when using FID, complete resolution is essential.

12.1.1 Calculate endothall concentration.

$$C_x = \frac{(A_x)(Q_{is})}{(A_{is})\ RF\ V}$$

where:

$C_x$ = concentration of endothall in µg/L in the water sample.

$A_x$ = integrated abundance of the quantitation ion of endothall (m/z 183) in the sample.
$A_{is}$ = integrated abundance of the quantitation ion of the internal standard (m/z 164) in the sample.
$Q_{is}$ = total quantity (in micrograms) of internal standard added to the water sample.
V = original water sample volume in liters.
RF = mean response factor endothall from the initial calibration.

12.1.2 Alternatively, use the GC/MS data system software or other available proven software to compute the concentration of the endothall from the linear calibration or the second order regression curves.

12.1.3 Calculations should utilize all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures (one digit of uncertainty). Experience indicates that three significant figures may be used for concentrations above 99 µg/L, two significant figures for concentrations between 1-99 µg/L, and one significant figure for lower concentrations.

## 13. METHOD PERFORMANCE

13.1 METHOD DETECTION LIMITS -- The MDL is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above the background level (1). The MDLs listed in Table 1 were obtained using reagent water for detection by GC/MS and FID.

13.2 In a single laboratory study on fortified reagent water and ground water matrices, the mean recoveries and relative standard deviations presented in Table 2 were obtained. Table 3 provides the concentrations of CaII, MgII and sulfate for two high ionic strength drinking water sources studied. Table 4 presents mean recovery data for these fortified sources with and without the addition of EDTA and/or sample dilution.

## 14. POLLUTION PREVENTION

14.1 This method utilizes the new liquid-solid extraction technology which requires the use of very little organic solvent thereby eliminating the hazards involved with the use of large volumes of organic solvents in conventional liquid-liquid extractions. It also uses acidic methanol as the derivatizing reagent in place of the highly toxic and explosive diazomethane. These features make this method much safer for the analyst to employ and a great deal less harmful to the environment.

## 15. WASTE MANAGEMENT

15.1 Due to the nature of this method, there is very little need for waste management. No large volumes of solvents or hazardous chemicals are used. The matrices are drinking water or source water, and can be discarded down the sink.

## 16. REFERENCES

1. 40 CFR Part 136, Appendix B.

2. ASTM Annual Book of Standards, Part 31, D3694-78. "Standard Practices for Preparation of Sample Containers and for Preservation of Organic Constituents," American Society for Testing and Materials, Philadelphia, PA.

3. "Carcinogens-Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.

4. "OSHA Safety and Health Standards, General Industry," (29CFR1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).

5. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

## 17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

### TABLE 1. RETENTION TIMES AND METHOD DETECTION LIMITS

| COMPOUND | RETENTION TIME, min. Column A | Column B | Column C | METHOD DETECTION LIMIT GC/MS µg/L[1] | FID |
|---|---|---|---|---|---|
| Endothall | 16.02 | 19.85 | 18.32 | 1.79 | 0.7 |
| d10-Acenaphthene | 14.69 | | | | |

[1] Based on 7 replicate analyses of a reagent water fortified at 2 µg/L

Column A: DB-5 fused silica capillary for GC/MS, 30 m x 0.25 mm, 0.25 micron film

MS inlet temperature = 200°C
Injector temperature = 200°C

Temperature Program: Hold 5 min at 80°C, increase to 260°C at 10°/min, hold 10 min.

Column B: FID primary column, RTX Volatiles, 30 m x 0.53 mm I.D., 2 micron film thickness.

Detector temperature = 280°C
Injector Temperature = 200°C

Carrier gas velocity = 50 cm/sec.

Temperature program: Same as Column A

Column C: FID confirmation column, DB-5, 30 m x 0.32 mm ID, 0.25 micron film

Carrier Gas velocity = 27 cm/sec

Same injector, detector and temperature program as Column A.

### TABLE 2. ENDOTHALL METHOD DEVELOPMENT DATA

| Matrix | Conc. µg/L | Recovery[1] % | RSD[2] % |
|---|---|---|---|
| Reagent Water | 2 | 101 | 10 |
| Reagent Water | 10 | 86 | 10 |
| Reagent Water | 100 | 95 | 3 |
| Ground Water[3] | 2 | 91 | 25 |
| Ground Water | 10 | 82 | 14 |
| Ground Water | 100 | 88 | 6 |

[1] Based on analysis of 7 replicates.

[2] Relative Standard Deviation.

[3] High Humic Content Florida Ground Water.

## TABLE 3. MATRIX ANALYSES[1]

| Major Ion | Western Surface, mg/L | Eastern Ground mg/L |
|---|---|---|
| Ca | 330 | 122 |
| Mg | 132 | 33 |
| Na | 400 | 23 |
| Sulfate | 1850 | 102 |

[1] Determination by inductively coupled plasma - mass spectrometry for cations and ion chromatography for sulfate.

## TABLE 4. ENDOTHALL METHOD VALIDATION DATA

| Matrix | Conc. µg/L | EDTA[1] Mole/L | Recovery[2] % | RSD % |
|---|---|---|---|---|
| WS[3] | 25 | 0 | 9 | 19 |
| WS - 1/10[4] | 50 | 0 | 66 | 13 |
| WS - 1/10 | 50 | 0.002 | 88 | 5 |
| EG[5] | 25 | 0 | 43 | 17 |
| EG | 25 | 0.005 | 97 | 6 |
| EG - 1/5 | 25 | 0 | 97 | 5 |

[1] Ethylenediamine Tetraacetic Acid

[2] Based on 7 Replicates

[3] WS - Treated Western Surface Water

[4] Dilution Factor in Reagent Water

[5] WG - Eastern Ground Water

## TABLE 5. ION ABUNDANCE CRITERIA FOR BIS(PERFLUOROPHENYL)PHENYL PHOSPHINE (DECAFLUOROTRIPHENYLPHOSPHINE, DFTPP)

| Mass (M/z) | Relative Abundance Criteria | Purpose of Checkpoint[1] |
|---|---|---|
| 51 | 10-80% of the base peak | low mass sensitivity |
| 68 | <2% of mass 69 | low mass resolution |
| 70 | <2% of mass 69 | low mass resolution |
| 127 | 10-80% of the base peak | low-mid mass sensitivity |
| 197 | <2% of mass 198 | mid-mass resolution |
| 198 | base peak or >50% of 442 | mid-mass resolution and sensitivity |
| 199 | 5-9% of mass 198 | mid-mass resolution and isotope ratio |
| 275 | 10-60% of the base peak | mid-high mass sensitivity |
| 365 | >1% of the base peak | baseline threshold |
| 441 | Present and < mass 443 | high mass resolution |
| 442 | base peak or >50% of 198 | high mass resolution and sensitivity |
| 443 | 15-24% of mass 442 | high mass resolution and isotope ratio |

[1] All ions are used primarily to check the mass measuring accuracy of the mass spectrometer and data system, and this is the most important part of the performance test. The three resolution checks, which include natural abundance isotope ratios, constitute the next most important part of the performance test. The correct setting of the baseline threshold, as indicated by the presence of low intensity ions, is the next most important part of the performance test. Finally, the ion abundance ranges are designed to encourage some standardization to fragmentation patterns.

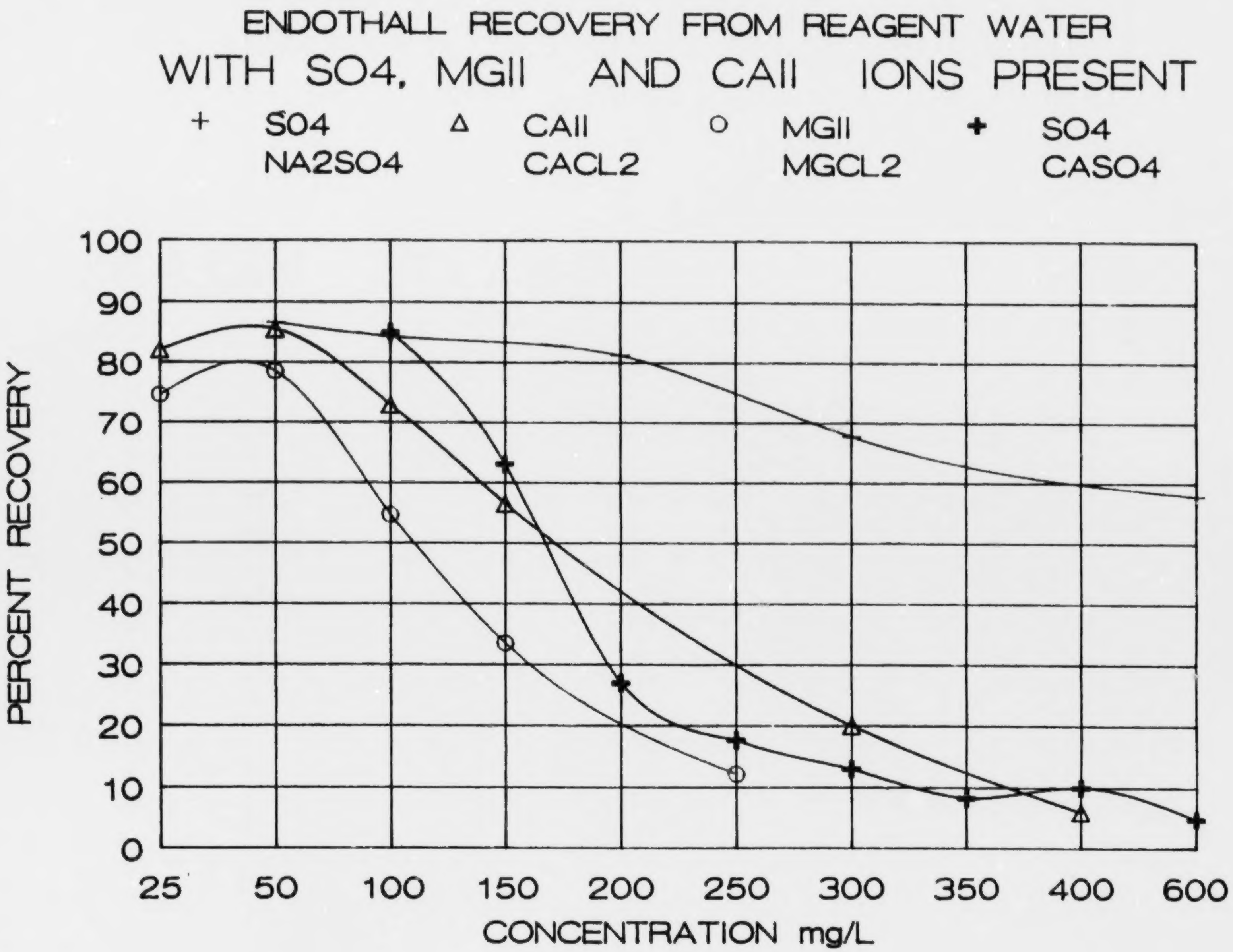


FIGURE 1. ENDOTHALL RECOVERY VERSUS ION CONCENTRATIONS

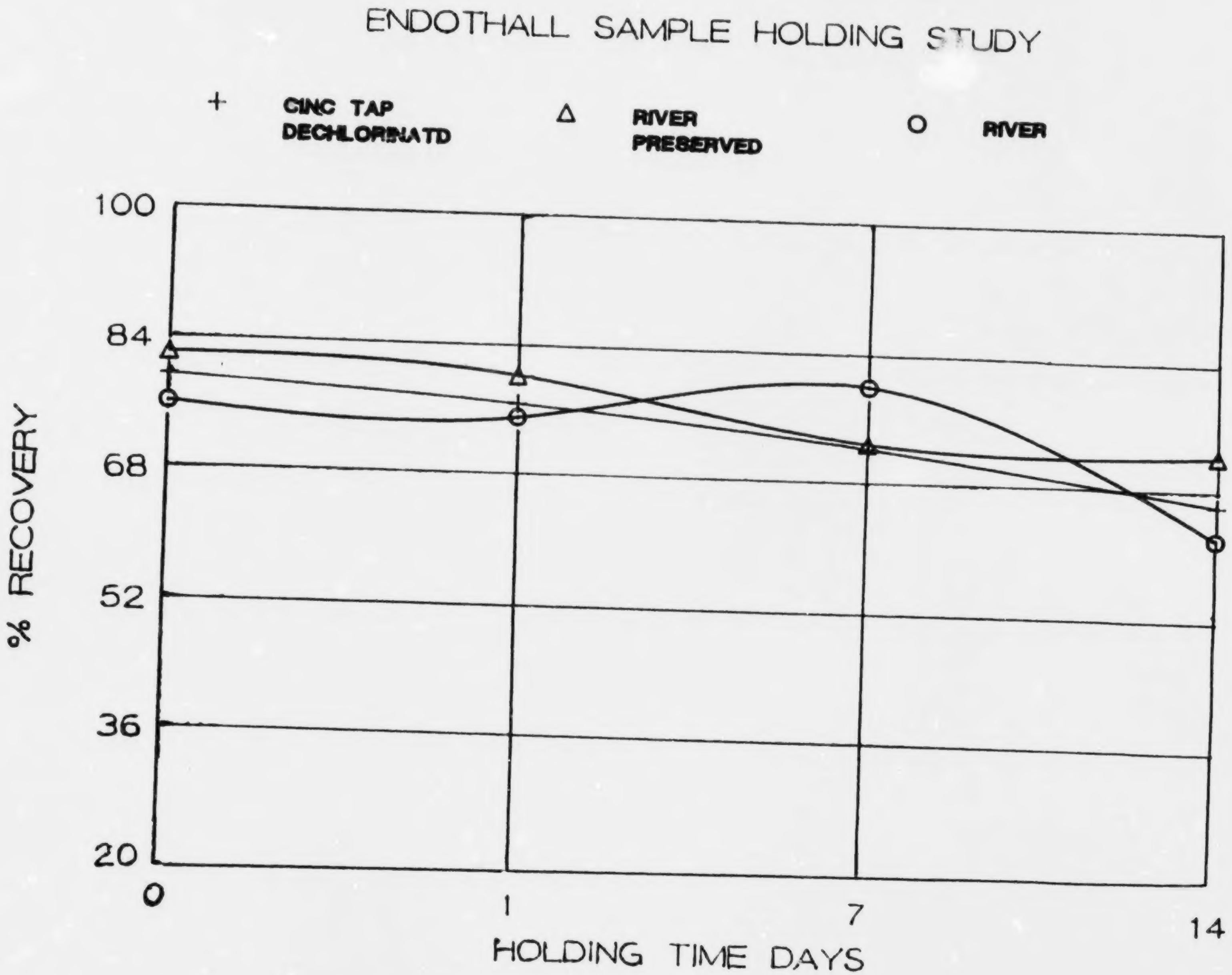


FIGURE 2. ENDOTHALL HOLDING STUDY

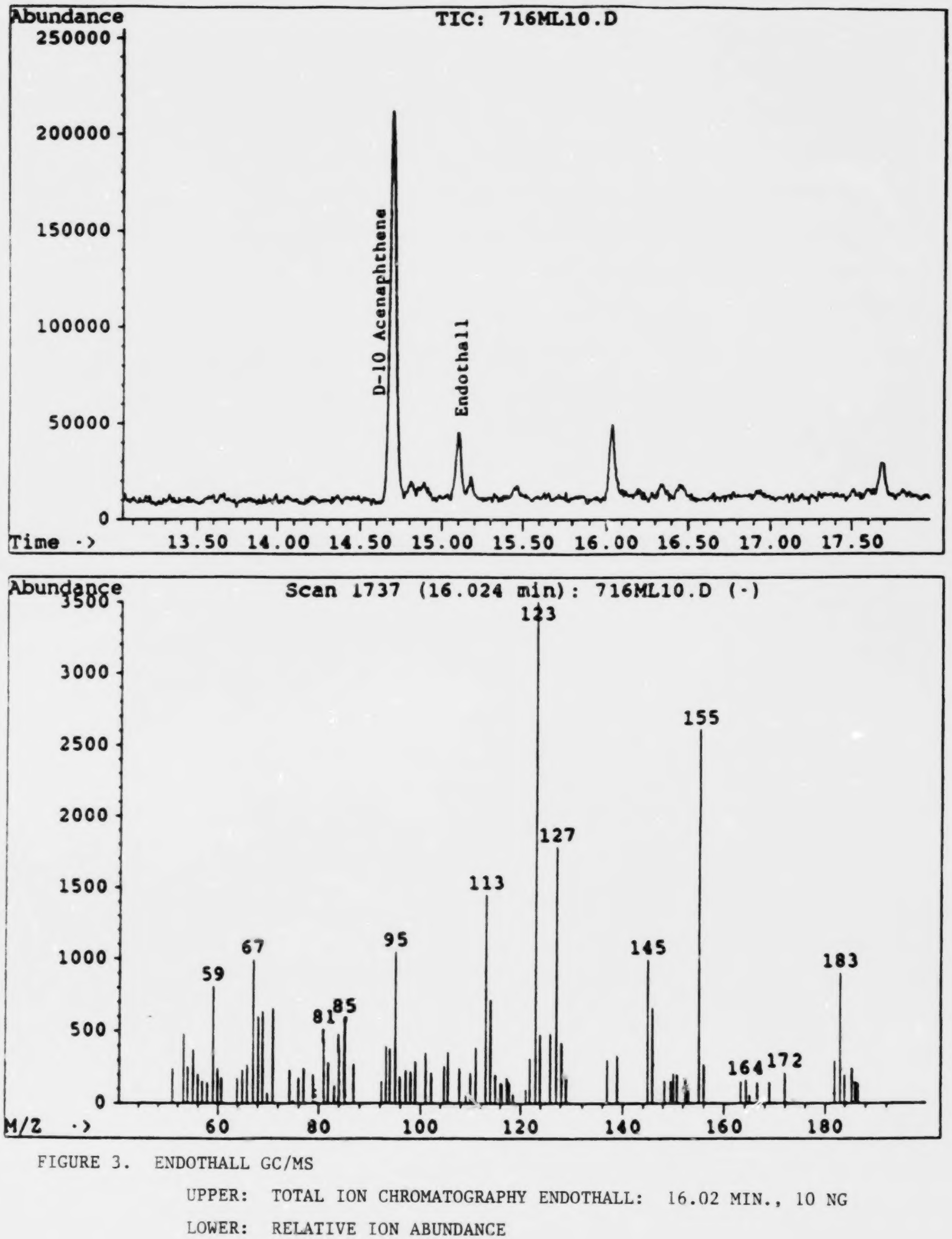


FIGURE 3. ENDOTHALL GC/MS

UPPER: TOTAL ION CHROMATOGRAPHY ENDOTHALL: 16.02 MIN., 10 NG

LOWER: RELATIVE ION ABUNDANCE

# METHOD 549.1 DETERMINATION OF DIQUAT AND PARAQUAT IN DRINKING WATER BY LIQUID-SOLID EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

Revision 1.0

August 1992

J. W. Hodgeson

W. J. Bashe (Technology Applications Inc.)

James W. Eichelberger

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

Preceding page blank

## METHOD 549.1

## DETERMINATION OF DIQUAT AND PARAQUAT IN DRINKING WATER BY LIQUID-SOLID EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

### 1. SCOPE AND APPLICATION

1.1 This is a high performance liquid chromatography (HPLC) method for the determination of diquat (1,1'-ethylene-2,2'-bipyridilium dibromide salt) and paraquat (1,1'-dimethyl-4,4'- bipyridilium dichloride salt) in drinking water sources and finished drinking water (1,2).

| Analytes | Chemistry Abstract Services Registry Number |
|---|---|
| Diquat | 85-00-7 |
| Paraquat | 1910-42-5 |

1.2 When this method is used to analyze unfamiliar samples, compound identification should be supported by at least one additional qualitative technique. The use of a photodiode array detector provides ultraviolet spectra that can be used for the qualitative confirmation.

1.3 The method detection limits (MDL, defined in Sect. 13) (3) for diquat and paraquat are listed in Table 1.

1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Sect. 9.3.

### 2. SUMMARY OF METHOD

2.1 A measured volume of liquid sample, approximately 250 mL, is adjusted to pH 10.5. The sample is extracted using a $C_8$ solid sorbent cartridge or a $C\text{-}_8$ disk which has been specially prepared for the reversed-phase, ion-pair mode. The disk or cartridge is eluted with 4.5 mL of an acidic aqueous solvent. After the ion-pair reagent is added to the eluate, the final volume is adjusted to 5.0 mL. Liquid chromatographic conditions are described which permit the separation and measurement of diquat and paraquat in the extract by absorbance detection at 308 nm and 257 nm, respectively. A photodiode array detector is utilized to provide simultaneous detection and confirmation of the method analytes (1,2).

2.2 Analysis of diquat and paraquat is complicated by their ionic nature. Glassware should be deactivated to prevent loss of analytes. The substitution of polyvinylchloride (PVC) for glass is recommended.

## 3. DEFINITIONS

3.1 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.2 FIELD REAGENT BLANK (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.3 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.4 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.5 STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.6 PRIMARY DILUTION STANDARD SOLUTION (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.7 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution and stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.8 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentration which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.9 EXTERNAL STANDARD (ES) -- A pure analyte(s) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard(s) is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the sample.

## 4. INTERFERENCES

4.1 Method interference may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatogram. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by analyzing laboratory reagent blanks as described in Sect. 9.2.

4.1.1 Glassware must be scrupulously cleaned (4). Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap water and distilled water. It should then be drained dry and heated in a laboratory oven at 130°C for several hours before use. Solvent rinses with methanol may be substituted for the oven heating. After drying and cooling, glassware should be stored in a clean environment to prevent any accumulation of dust or other contaminants.

4.1.2 Before the initial use of all glassware, the procedure described in Sect. 4.1.1 should be followed. Silanization of all glassware which will come in contact with the method analytes is necessary to prevent adsorption of the diquat and paraquat cations onto glass surfaces (7.13).

4.1.3 Plasticware should be washed with detergent and rinsed in tap water and distilled water. It should be drained dry before use.

4.1.4 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

4.2 Interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source. Because of the selectivity of the detection system used here, no interferences have been observed in the matrices studied. If interferences occur, some additional cleanup may be necessary.

## 5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be minimized. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis.

## 6. EQUIPMENT AND SUPPLIES

6.1 SAMPLING EQUIPMENT, discrete or composite sampling.

6.1.1 Grab sample bottle -- Amber polyvinylchloride (PVC) high density, 1-L, fitted with screw caps. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with deionized water, and dried before use to minimize contamination.

6.2 GLASSWARE

6.2.1 Volumetric flask -- 5 mL, silanized

6.2.2 Autosampler vials -- 4 mL, silanized

6.3 BALANCE -- analytical, capable of accurately weighing 0.0001 g

6.4 pH METER -- capable of measuring pH to 0.1 units

6.5 HPLC APPARATUS

6.5.1 Isocratic pumping system, constant flow (Waters M6000A HPLC pump or equivalent).

6.5.2 Manual injector or automatic injector, capable of delivering 200 μL.

6.5.3 Analytical column -- any column which produces results equal to or better than those listed below may be used.

6.5.3.1 Hamilton PRP-1, (5 µm, 150 mm x 4.1 mm), or equivalent.

6.5.3.2 Guard column, $C_8$ packing

6.5.4 Column Oven (Fiatron, Model CH-30 and controller, Model TC-50, or equivalent).

6.5.5 Photodiode array detector (LKB 2140 Rapid Spectral Detector or equivalent). Any detector which has the capability to switch between 257 nm and 308 nm may be used.

6.5.6 Data system -- Use of a data system to report retention times and peak areas is recommended but not required.

6.6 EXTRACTION APPARATUS

6.6.1 Liquid solid extraction cartridges, $C_8$, 500 mg or equivalent.

6.6.2 Liquid solid extraction system (Baker - 10 SPE, or equivalent).

6.6.3 Liquid solid extraction disks (C-8 Empore, 47 mm, or equivalent).

6.6.4 Liquid solid extraction system, Empore, 47 mm, 6 position manifold (Varian Associates or equivalent). If a disk extraction manifold is not used, all glassware used instead should be silanized or substituted with polypropylene ware.

6.6.5 Vacuum pump, 100 VAC, capable of maintaining a vacuum of 8-10 mm of Hg.

6.6.6 Membrane Filters, 0.45 µm pore-size, 47 mm diameter, Nylon.

## 7. REAGENTS AND STANDARDS

7.1 DEIONIZED WATER -- Water which has been processed through a series of commercially available filters including a particulate filter, carbon bed, ion exchange resin and finally a bacterial filter to produce deionized, reagent grade water. Any other source of reagent water may be used provided the requirements of Sect. 9 are met.

7.2 METHANOL -- HPLC grade or higher purity

7.3 ORTHOPHOSPHORIC ACID, 85% (w/v) -- Reagent grade

7.4 DIETHYLAMINE -- Reagent grade

7.5 CONCENTRATED SULFURIC ACID -- ACS reagent grade

7.6 SODIUM HYDROXIDE -- Reagent grade

7.7 CONCENTRATED HYDROCHLORIC ACID, 12 N -- Reagent grade

7.8 CETYL TRIMETHYL AMMONIUM BROMIDE, 95% -- Aldrich Chemical

7.9 SODIUM THIOSULFATE -- Reagent grade

7.10 1-HEXANESULFONIC ACID, sodium salt, 98%, Aldrich Chemical

7.11 1-HEPTANESULFONIC ACID, sodium salt, 98%, Aldrich Chemical

7.12 AMMONIUM HYDROXIDE, ACS, Concentrated

7.13 SYLON CT -- Silanization solution, Supelco

7.14 REAGENT SOLUTIONS

7.14.1 Conditioning solution A -- Dissolve 0.500 g of cetyl trimethyl ammonium bromide and 5 mL of concentrated ammonium hydroxide in 500 mL of deionized water and dilute to 1000 mL in volumetric flask.

7.14.2 Conditioning solution B -- Dissolve 10.0 g of 1-hexanesulfonic acid, sodium salt and 10 mL of concentrated ammonium hydroxide in 250 mL of deionized water and dilute to 500 mL in volumetric flask.

7.14.3 Sodium hydroxide solution, 10% w/v -- Dissolve 50 g of sodium hydroxide into 400 mL of deionized water and dilute to 500 mL in a volumetric flask.

7.14.4 Hydrochloric acid, 10% v/v -- Add 50 mL of concentrated hydrochloric acid to 400 mL of deionized water and dilute to 500 mL in a volumetric flask.

7.14.5 Disk or cartridge eluting solution -- Add 13.5 mL of orthophosphoric acid and 10.3 mL of diethylamine to 500 mL of deionized water and dilute to 1000 mL in a volumetric flask.

7.14.6 Ion-pair concentrate -- Dissolve 3.75 g of 1-hexanesulfonic acid in 15 mL of the disk or cartridge eluting solution and dilute to 25 mL in a volumetric flask with the disk eluting solution.

### 7.15 STOCK STANDARD SOLUTIONS

7.15.1 Diquat dibromide and Paraquat dichloride.

7.15.2 Stock diquat and paraquat solutions (1000 mg/L). Dry diquat and paraquat salts in an oven at 110°C for 3 hr. Cool in a desiccator. Repeat process to a constant weight. Weigh 0.1968 g of dried diquat salt and 0.1770 g of dried paraquat salt and place into a silanized glass or polypropylene 100-mL volumetric flask. Dissolve with approximately 50 mL of deionized water. Dilute to the mark with deionized water.

7.15.3 The salts used in preparing the stock standards (Sect. 7.15.2) were taken to be diquat dibromide monohydrate and paraquat dichloride tetrahydrate (5). The drying procedure described in Sect. 7.15.2 will provide these hydration levels.

7.16 MOBILE PHASE -- Make mobile phase by adding the following to 500 mL of deionized water: 13.5 mL of orthophosphoric acid; 10.3 mL of diethylamine; 3.0 g of 1-hexanesulfonic acid, sodium salt. Mix and dilute with deionized water to a final volume of 1 L.

## 8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Grab samples must be collected in either amber PVC high density bottles or silanized amber glass bottles. Conventional sampling procedures should be followed (6). Automatic sampling equipment must be free as possible of adsorption sites which might extract the sample.

8.2 The samples must be iced or refrigerated at approximately 4°C from the time of collection until extraction. The analytes are light-sensitive, particularly diquat.

8.3 Samples which are known or suspected to contain residual chlorine must be preserved with sodium thiosulfate (100 mg/L). Samples which are biologically active must be preserved by adding sulfuric acid to pH 2 to prevent adsorption of method analytes by the humectant material.

8.4 Analyte stability over time may depend on the matrix tested. Analyte stability in representative drinking water matrices is listed in Table 3. All samples must be extracted within 7 days of collection. Extracts must be analyzed within 21 days of extraction (1).

## 9. QUALITY CONTROL

9.1 Minimum quality control (QC) requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified matrix samples, and laboratory fortified blanks. The laboratory must maintain records to document the quality of the data generated. Additional quality control practices are recommended.

9.2 LABORATORY REAGENT BLANKS (LRB) -- Before processing any samples, the analyst must analyze a LRB to demonstrate that all deactivated glassware or plasticware, and reagent interferences are reasonably free of contamination. In addition, each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window (Sect. 11.3.2) of the analyte of interest, the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

9.3 INITIAL DEMONSTRATION OF CAPABILITY

9.3.1 Prepare laboratory fortified blanks (LFBs) at analyte concentrations of 100 µg/L. With a syringe, add 25 µL of the stock standard (Sect. 7.14.2) to at least four 250 mL aliquots of reagent water and analyze each aliquot according to procedures beginning in Sect. 11.2.

9.3.2 Calculate the recoveries, relative standard deviation (RSD), and the MDL (3). The recovery (R) values should be within ± 30% of the R values listed in Table 2 for at least three of four consecutive samples. The RSD of the mean recovery should be less than 30%. The MDL must be sufficient to meet the requirements of the SDWA regulations. For analytes that fail this critera, initial demonstration procedures should be repeated.

9.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. As laboratory personnel gain experience with this method the quality of the data should improve beyond the requirements stated in Sect. 9.3.2.

9.4 The analyst is permitted to use other HPLC columns, HPLC conditions, or detectors to improve separations or lower analytical costs. Each time such method modifications are made, the analyst must repeat the procedures in Sect. 9.3.

## 9.5 LABORATORY FORTIFIED BLANKS

9.5.1 The laboratory must analyze at least one laboratory fortified blank (LFB) sample per sample set (all samples extracted within a 24-hr period). The fortified concentration of each analyte in the LFB should be 10 times the MDL. If the recovery of either analyte falls outside the control limits (Sect. 9.5.2), that analyte is judged out of control, and the source of the problem must be identified and resolved before continuing analyses.

9.5.2 Until sufficient data become available, usually a minimum of results from 20 to 30 analyses, the laboratory should assess laboratory performance against the control limits in Sect. 9.3.2. When sufficient internal performance data become available, develop control limits from the mean percent recovery (R) and standard deviation ($S_r$) of the percent recovery. These data are used to establish upper and lower control limits as follows:

UPPER CONTROL LIMIT = $R + 3S_r$
LOWER CONTROL LIMIT = $R - 3S_r$

After each five to ten new recovery measurements, new control limits should be calculated using only the most recent 20-30 data points.

## 9.6 LABORATORY FORTIFIED SAMPLE MATRIX

9.6.1 The laboratory must add a known fortified concentration to a minimum of 10% of the samples or one fortified sample per set, whichever is greater. The fortified concentration should not be less than the background concentration of the original sample. Ideally, the fortified concentration should be the same as that used for the laboratory fortified blank (Sect. 9.5). Over time, samples from all routine samples sources should be fortified.

9.6.2 Calculate the accuracy as percent recovery (R) for each analyte, corrected for background concentrations measured in the original sample, and compare these values to the control limits established in Sect. 9.5.2 from the analyses of LFBs.

9.6.3 If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Sect. 9.5), the recovery problem encountered with the dosed sample is judged to be matrix related, not system related. The result for that analyte in the original sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.7 QUALITY CONTROL SAMPLES (QCS) -- Each quarter the laboratory should analyze one or more QCS. If criteria provided with the QCS are not met, corrective action should be taken and documented.

9.8 The laboratory may adopt additional quality control practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage.

## 10. CALIBRATION AND STANDARDIZATION

10.1 Establish HPLC operating conditions indicated in Table 1. The chromatographic system can be calibrated using the external standard technique.

10.2 In order to closely match calibration standards to samples, process standards by the following method: Using $C-_8$ disks or $C_8$ cartridges conditioned according to Sect. 11.2.1, pass 250 mL of reagent water through the disk or cartridge and discard the water. Dry the disk or cartridge by passing 5 mL of methanol through it. Discard the methanol. Pass 4.0 mL of the eluting solution through the disk or cartridge and catch in a 5 mL silanized volumetric flask. Fortify the eluted solution with 100 $\mu$L of the ion-pair concentrate and with 500 $\mu$L of the stock standard and dilute to the mark with eluting solution. This provides a 10:1 dilution of the stock. Use serial dilution of the calibration standard by the same method to achieve lower concentration standards.

10.3 Analyze a minimum of three calibration standards prepared by the procedure described in Sect. 10.2 utilizing the HPLC conditions given in Table 1. From full spectral data obtained, extract the 308 nm chromatographic trace for diquat and the 257 nm trace for paraquat. Integrate and record the analyte peak areas. Any mathematical manipulations performed to aid in data reduction must be recorded and performed on all sample chromatograms. Tabulate the peak area against quantity injected. The results may be used to prepare calibration curves for diquat and paraquat.

10.4 The working calibration curve must be verified on each working day by measurement of a calibration check standard, at the beginning of the analysis day. These check standards should be at two different concentration levels to verify the calibration curve. For extended periods of analysis (greater than 8 hr), it is strongly recommended that check standards be interspersed with samples at regular intervals. If the response for any analyte varies from the predicted response by more than ±20%, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a new calibration curve.

## 11. PROCEDURE

11.1 SAMPLE CLEANUP -- Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various sample types. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must demonstrate that the recovery of the analytes is within the limits specified by the method.

11.1.1 If the sample contains particulates, or the complexity is unknown, the entire sample should be passed through a 0.45 μm Nylon membrane filter into a silanized glass or plastic container.

11.1.2 Store all samples at 4°C unless extraction is to be performed immediately.

11.2 CARTRIDGE EXTRACTION

11.2.1 Before sample extraction, the C-8 extraction cartridges must be conditioned by the following procedure.

11.2.1.1 Place a $C_8$ cartridge on the cartridge extraction system manifold.

11.2.1.2 Elute the following solutions through the cartridge in the stated order. Take special care not to let the column go dry. The flow rate through the cartridge should be approximately 10 mL/min.

11.2.1.2.1 Cartridge Conditioning Sequence

a. Deionized water, 5 mL
b. Methanol, 5 mL
c. Deionized water, 5 mL
d. Conditioning Solution A, 5 mL
e. Deionized water, 5 mL
f. Methanol, 10 mL
g. Deionized water, 5 mL
h. Conditioning Solution B, 10 mL

11.2.1.2.2 Retain conditioning solution B in the $C_8$ cartridge to keep it activated.

11.2.2 The $C_8$ cartridges should not be prepared more than 48 hr prior to use. After conditioning, the cartridge should be capped and stored at 4°C.

11.2.3 Measure a 250-mL aliquot of the sample processed through Sect. 11.1 in a silanized, volumetric flask.

11.2.4 Immediately before extraction, adjust the pH of sample to 10.5 ± 0.2 with 10% w/v NaOH (aq) or 10% v/v HCl (aq).

11.2.5 Place a conditioned $C_8$ cartridge on the solid phase extraction vacuum manifold. Attach a 60-mL reservoir to the $C_8$ cartridge with the appropriate adapter. Put a 250-mL beaker inside the extraction manifold to catch waste solutions and sample. Transfer the measured volume in aliquots to the reservoir. Turn on the vacuum pump or house vacuum and adjust the flow rate to 3 to 6 mL/min. Filter the sample through the $C_8$ cartridge, and wash the column with 5 mL of HPLC grade methanol. Continue to draw the vacuum through the cartridge for one additional minute to dry the cartridge. Release the vacuum and discard the sample waste and methanol.

11.2.6 Place a silanized 5-mL volumetric flask beneath the collection stem in the vacuum manifold. Add 4.5 mL of the eluting solution to the sample cartridge. Turn on the vacuum and adjust the flow rate to 1 to 2 mL/min.

11.2.7 Remove the 5-mL volumetric flask with the extract. Fortify the extract with 100 µL of the ion-pair concentrate. Adjust the volume to the mark with cartridge eluting solution, mix thoroughly, and seal tightly until analyzed.

11.2.8 Analyze sample by HPLC using conditions described in Table 1. Integration and data reduction must be consistent with that performed in Sect. 10.3. Figure 1 presents a representative, sample chromatogram.

11.3 DISK EXTRACTION -- The top surface of the disk matrix must remain covered with liquid at all times. If the disk is exposed to air at any step in the disk cleanup procedure, the elution procedure should be restarted. Eluants applied to the disk should be allowed to soak into the disk before drawing them through. Vacuum should then be applied to draw most of the eluant through the disk, leaving a thin layer of solution on the top of the disk. Flow rate through the disk is not critical.

11.3.1 Assemble the 47 mm disk in the disk holder or a filter apparatus. Be sure that the surfaces of the holder are either glass or Teflon coated to avoid adsorption or decomposition of the analytes.

11.3.2 Adjust the pH of the sample to 10.5 ± 0.2 with 10% w/v aqueous sodium hydroxide or 10% v/v aqueous hydrochloric acid solution. Once the pH has been adjusted, the steps below must be performed immediately.

11.3.3 Apply 10 mL of methanol to the disk. Apply vacuum to begin elution, then immediately vent the vacuum when drops of liquid appear at the drip tip. Allow the methanol to soak into the disk for a minimum of 1 min, then reapply the vacuum to bring the methanol to just above the top surface of the disk.

11.3.4 Draw 2 10-mL aliquots of reagent water through to just above the top surface of the disk to remove the methanol.

11.3.5 Apply 10 mL of Conditioning Solution A to the disk. As with the methanol, draw a few drops through, then allow the disk to soak for at least 1 min. Draw the Conditioning Solution A through the disk to just above its top surface.

11.3.6 Draw 2 10-mL aliquots of reagent water through to just above the top surface of the disk.

11.3.7 Apply 10 mL of Conditioning Solution B to the disk. Draw a few drops through using vacuum and allow the disk to soak for at least 1 min. Draw the remaining Conditioning Solution B through to just above the top surface of the disk.

11.3.8 Measure 250 mL of the pH adjusted sample using a polypropylene graduated cylinder. Pour the sample aliquot into the filtration apparatus reservoir and apply vacuum to draw the sample through the disk. Pass the entire sample through the disk, leaving no liquid on the top of the disk, then vent the vacuum.

11.3.9 Assemble a graduated collection tube under the drip tip with the tip descending into the tube slightly to prevent losses of eluants. Be sure the tube will hold at least 10 mL of eluate.

11.3.10 With the vacuum vented, drip enough methanol onto the disk to cover it completely (0.5-1.0 mL). Allow the methanol to soak into the disk for 1 min. Add more methanol as needed to keep the disk covered as it soaks.

11.3.11 Pipet 4 mL of Disk Eluting Solvent onto the disk. Apply vacuum until drops appear at the drip tip. Vent the vacuum and allow the disk to soak for 1 min.

11.3.12 Draw the Disk Eluting Solution through to just above the top surface of the disk. Add 4 mL of Disk Eluting

Solution and draw it completely through the disk. Tap the disk holder assembly gently to loosen adhering drops into the collection tube.

11.3.13 Vent the vacuum, disassemble the disk extraction device, and remove the collection tube. Add disk elution solution to the tube to a known volume.

11.3.14 Analyze samples by HPLC. Some suggested conditions, which were used in developing this method, are listed in Table 1. This table includes the retention times and MDLs that were obtained using the suggested conditions. Figure 1 displays a representative sample chromatogram.

### 11.4 IDENTIFICATION OF ANALYTES

11.4.1 Identify a sample component by comparison of its retention time to the retention time of a reference chromatogram. If the retention time of an unknown compound corresponds, within limits (Sect. 11.4.2), to the retention time of a standard compound, then identification is considered positive.

11.4.2 The width of the retention time window used to make identification should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.4.3 Identification requires expert judgment when sample components are not resolved chromatographically. When peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima), or any time doubt exists over the identification of a peak in a chromatogram, a confirmatory technique must be employed. Through the use of the photodiode array detector, full spectra of the analyte peaks are obtained (Figure 2). When a peak of an unknown sample falls within the retention time windows of method analytes, confirm the peak identification by spectral comparison with analyte standards.

If additional confirmation is required, replace the 1-hexanesulfonic acid salt with 1-heptanesulfonic acid, sodium salt in the mobile phase and reanalyze the samples. Comparison of the ratio of retention times in the samples by the two mobile phases with that of the standards will provide additional confirmation.

11.4.4 If the peak area exceeds the linear range of the calibration curve, a smaller sample volume should be used. Alternatively, the final solution may be diluted with mobile phase and reanalyzed.

## 12. DATA ANALYSIS AND CALCULATIONS

12.1 Determine the concentration of the analytes in the sample.

12.1.1 Calculate the concentration of each analyte injected from the peak area using the calibration curves in Sect. 10.3 and the following equation.

$$\text{Concentration, } \mu g/L = \frac{(A) \times (VF)}{(VS)}$$

where:
A = Peak area of analyte in sample extract
VF = Final volume of sample extract, in mL
VS = Sample volume, in mL

12.2 Report results as micrograms per liter without correction for recovery data. When duplicate and fortified samples are analyzed, report all data obtained with sample results.

## 13. METHOD PERFORMANCE

13.1 METHOD DETECTION LIMITS -- The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above the background level (3). The MDL data listed in Table 1 were obtained using both cartridges and disks with reagent water as the matrix.

13.2 This method has been tested for linearity of recovery from fortified reagent water and has been demonstrated to be applicable over the range from 4 x MDL to 1000 x MDL.

13.3 Single-laboratory precision and accuracy results at several concentration levels in drinking water matrices using cartridges are presented in Table 2A. Single laboratory accuracy and precision data at a low, a medium, and a fairly high concentration of each compound in several matrices are listed in Table 2B.

## 14. POLLUTION PREVENTION

14.1 Only an extremely small volume of an organic solvent is used in this method. A maximum of 15 mL of methanol is used per sample to condition each cartridge or disk. Methanol is not considered to be a toxic or hazardous solvent. All other chemicals used in this method are not considered toxic when used in the prescribed amounts.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

## 15. WASTE MANAGEMENT

15.1 There are generally no waste management problems involved with discarding spent or left over samples in this method since most often the sample matrix is drinking water. If a sample is analyzed which appears to be highly contaminated with chemicals, analyses should be carried out to assess the type and degree of contamination so that the samples may be discarded properly. The Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge restrictions. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" also available from the American Chemical Society at the address in Sect. 14.2.

## 16. REFERENCES

1. Bashe, W. J., "Determination of Diquat and Paraquat in Drinking Waters by High Performance Liquid Chromatography with Ultraviolet Detection," final report, U.S. Environmental Protection Agency, March, 1989.

2. Lagman, L. [illegible]. and J. R. Hale, "Analytical Method for the Determination of Diquat in Aquatic Weed Infested Lakes and Rivers in South Carolina," Technology Conference Proceedings, WQTC-15, American Water Works Association, November 15-20, 1987.

3. Glaser, J. A., D. L. Foerst, G. M. McKee, S. A. Quave, and W. L. Budde, "Trace Analyses for Wastewaters," Environ. Sci. Technol., 15, 1426, 1981.

4. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Container and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.

5. Worobey, B. L., "Analytical Method for the Simultaneous Determination of Diquat and Paraquat Residues in Potatoes by High Pressure Liquid Chromatography," Pestic. Sci 18(4), 245, 1987.

6. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.

7. "Handbook of Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.

## 17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

### TABLE 1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY CONDITIONS AND METHOD DETECTION LIMITS

| Analyte | Retention Time (min) | Method Detection Limits[a] (µg/L) (cartridges) | Method Detection Limits[b] (µg/L) (disks) |
|---|---|---|---|
| Diquat | 2.1 | 0.44 | 0.51 |
| Paraquat | 2.3 | 0.80 | 0.59 |

HPLC Conditions:

| | |
|---|---|
| Column: | Hamilton PRP-1, 5µ, 4.1 mm x 150 mm |
| Column Temperature: | 35.0 C |
| Flow Rate: | 2.0 mL/min., Ion-Pair Mobile Phase (Sect. 7.16) |
| Injection Volume: | 200 µL |

Photodiode Array Detector Settings:

| | |
|---|---|
| Wavelength Range: | 210 - 370 nm |
| Sample Rate: | 1 scan/sec. |
| Wavelength Step: | 1 nm |
| Integration Time: | 1 sec. |
| Run Time: | 5.0 min. |

Quantitation

| | |
|---|---|
| Wavelengths: | Diquat - 308 nm<br>Paraquat - 257 nm |

---

[a] MDL data were obtained from six samples fortified at 2 µg/L diquat and 2.3 µg/L paraquat.

[b] MDL data were obtained from eight samples fortified at 1 µg/L diquat and 1 µg/L paraquat.

## TABLE 2A. SINGLE OPERATOR ACCURACY AND PRECISION USING CARTRIDGES

| Analyte | Matrix Type | Number of Analyses | Fortified Concentration µg/L | Relative Accuracy (Recovery) % | Relative Standard Deviation % |
|---|---|---|---|---|---|
| Diquat | Reagent Water | 6 | 2.0 | 85.6 | 5.1 |
| | | 6 | 10 | 92.1 | 7.3 |
| | | 7 | 100 | 96.2 | 5.6 |
| | | 7 | 1000 | 90.0 | 9.8 |
| | Ground Water | 6 | 100 | 102. | 3.7 |
| | Tap[a] Water | 6 | 100 | 91.3 | 4.7 |
| Paraquat | Reagent Water | 6 | 2.3 | 87.6 | 9.1 |
| | | 7 | 11 | 99.7 | 6.9 |
| | | 7 | 113 | 94.4 | 12. |
| | Ground Water | 6 | 113 | 92.1 | 3.4 |
| | Tap[a] Water | 6 | 113 | 74.2 | 1.8 |

[a] Dechlorinated with $Na_2S_2O_3$ (100 mg/L)

## TABLE 2B. SINGLE OPERATOR ACCURACY AND PRECISION USING DISK (N = 5 FOR EACH TYPE OF WATER)

DIQUAT

| Type of Water | Fortified 2.62 µg/L | | Fortified 10.5 µg/L | | Fortified 52.5 µg/L | |
|---|---|---|---|---|---|---|
| | Mean % Rec. | % RSD | Mean % Rec. | % RSD | Mean % Rec. | % RSD |
| RW | 99.2 | 7.0 | 88.0 | 2.5 | 83.8 | 5.9 |
| DW | 93.5 | 5.4 | 82.2 | 4.1 | 84.1 | 2.2 |
| GW | 87.4 | 5.8 | 90.3 | 4.6 | 90.5 | 1.4 |
| SW | 86.5 | 4.2 | 82.4 | 5.6 | 87.4 | 6.5 |
| WW | 90.5 | 7.1 | 78.0 | 5.2 | 86.1 | 3.4 |

PARAQUAT

| Type of Water | Fortified 2.47 µg/L | | Fortified 9.9 µg/L | | Fortified 49.5 µg/L | |
|---|---|---|---|---|---|---|
| | Mean % Rec. | % RSD | Mean % Rec. | % RSD | Mean % Rec. | % RSD |
| RW | 94.7 | 9.9 | 92.6 | 4.7 | 90.1 | 1.4 |
| DW | 100.1 | 6.1 | 85.2 | 4.1 | 85.7 | 1.2 |
| GW | 86.4 | 6.4 | 81.4 | 3.2 | 86.1 | 4.0 |
| SW | 77.4 | 8.9 | 71.8 | 5.3 | 81.0 | 2.6 |
| WW | 85.9 | 6.4 | 81.6 | 6.1 | 87.2 | 2.3 |

## TABLE 3. 14-DAY SAMPLE HOLDING/PRESERVATION DATA[a]

| | | Percent Recovery | | | | | |
|---|---|---|---|---|---|---|---|
| Analyte | Matrix | Day 0 | | Day 7 | | Day 14 | |
| | | R | $S_R$ | R | $S_R$ | R | $S_R$ |
| Diquat | RW[b] | 98.8 | ± 8.6 | 93.2 | ± 1.4 | 102. | ± 2.9 |
| | TW[c] | 84.1 | ± 1.0 | 94.1 | ± 5.8 | 94.4 | ± 12.0 |
| | GW[d] | 84.9 | ± 6.6 | 87.5 | ± 3.1 | 72.4 | ± 4.5 |
| Paraquat | RW | 90.8 | ± 4.4 | 86.8 | ± 4.4 | 89.2 | ± 3.9 |
| | TW | 72.1 | ± 0.8 | 86.7 | ± 4.7 | 84.7 | ± 2.9 |
| | GW | 98.1 | ± 1.4 | 72.5 | ± 4.8 | 66.4 | ± 7.9 |

[a] Average of four samples for each matrix. All matrices were preserved with $H_2SO_4$ (pH = 2). Concentration of each analyte was 100 µg/L.

[b] RW = Reagent Water

[c] TW = Tap Water - Dechlorinated with $Na_2S_2O_3$ (100 mg/L)

[d] GW = Groundwater

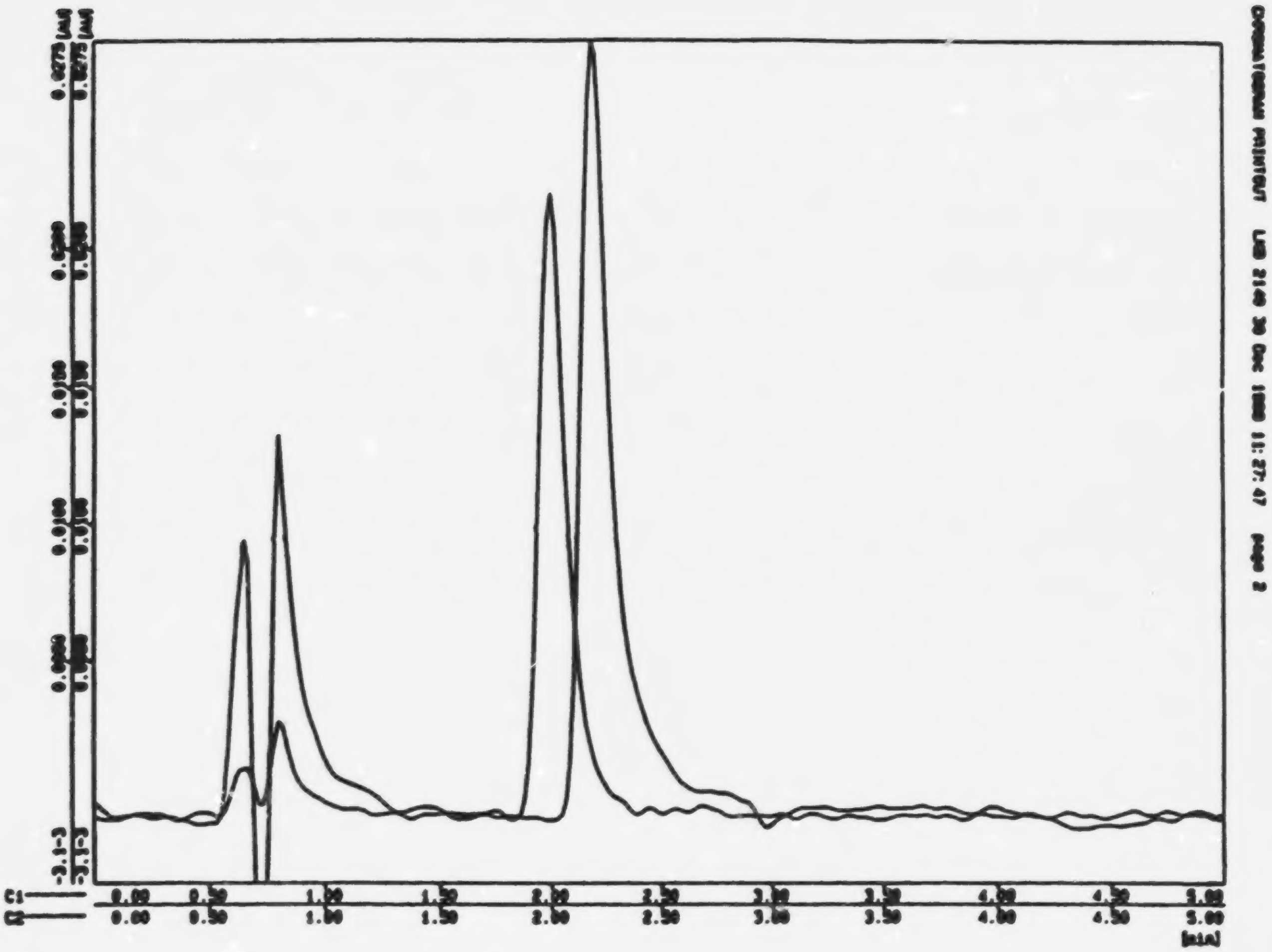


Figure 1. HPLC sample chromatograms of diquat (λ= 308 nm) and paraquat (λ= 257 nm). Retention time of diquat (C = 10 ug/L) is 2.03 min.; retention time of paraquat (C = 11 ug/L) is 2.25 min.

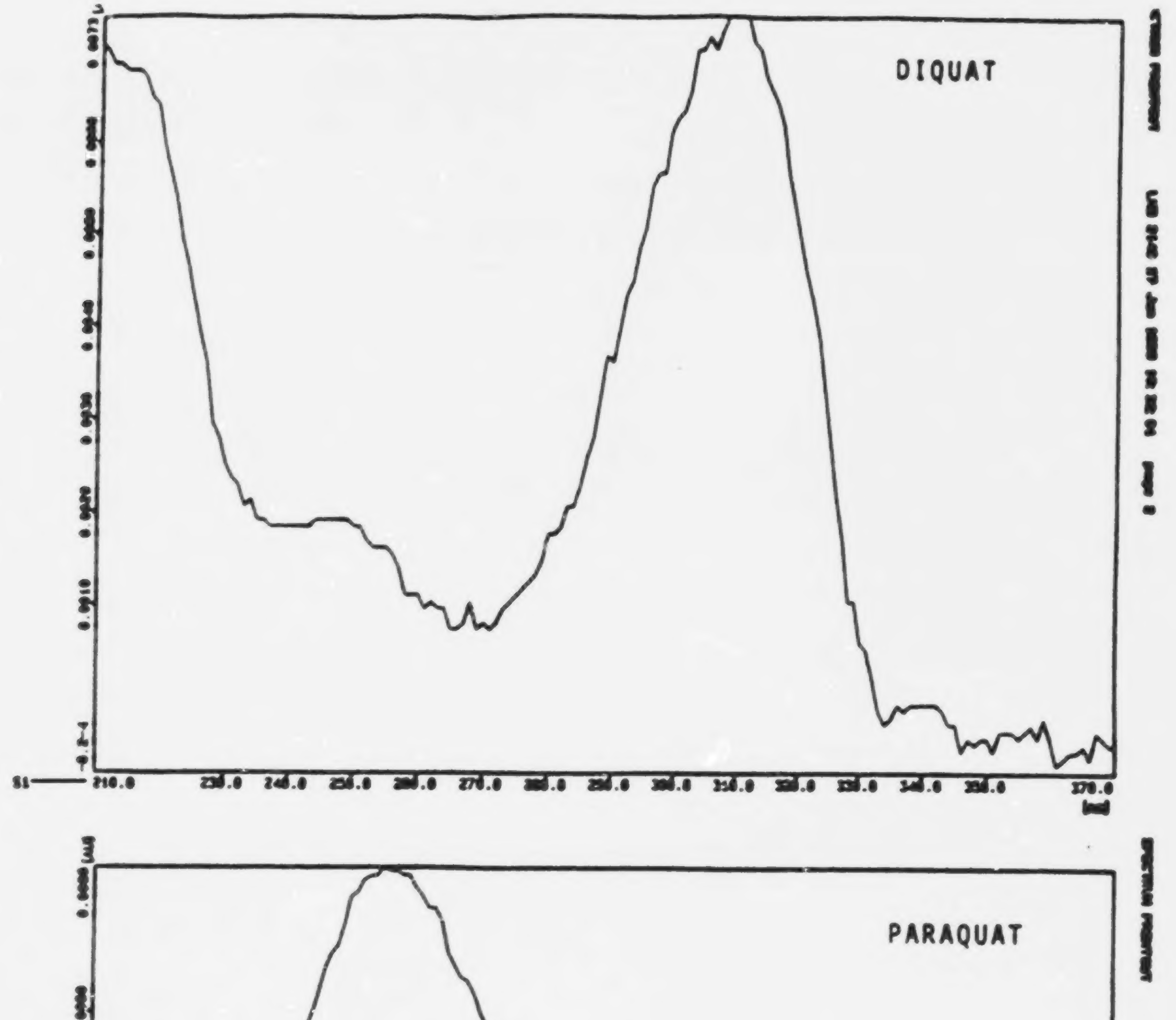


Figure 2. UV spectra of diquat at 10 ug/L and paraquat at 11 ug/L.

Reproduced from best available copy.

# METHOD 552.1 DETERMINATION OF HALOACETIC ACIDS AND DALAPON IN DRINKING WATER BY ION-EXCHANGE LIQUID-SOLID EXTRACTION AND GAS CHROMATOGRAPHY WITH AN ELECTRON CAPTURE DETECTOR

Revision 1.0

August 1992

Jimmie W. Hodgeson

David Becker (Technology Applications Inc.)

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

# METHOD 552.1

## DETERMINATION OF HALOACETIC ACIDS AND DALAPON IN DRINKING WATER BY ION-EXCHANGE LIQUID-SOLID EXTRACTION AND GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION

### 1. SCOPE AND APPLICATION

1.1 This is a gas chromatographic (GC) method (1) applicable to the determination of the listed halogenated acetic acids in drinking water, ground water, raw water and water at any intermediate treatment stage. In addition, the chlorinated herbicide, Dalapon, is determined using this method.

| Analyte | Chemical Abstract Services Registry Number |
|---|---|
| Monochloroacetic Acid | 79-11-8 |
| Dichloroacetic Acid | 79-43-6 |
| Trichloroacetic Acid | 76-03-9 |
| Monobromoacetic Acid | 79-08-3 |
| Bromochloroacetic Acid | 5589-96-3 |
| Dibromoacetic Acid | 631-64-1 |
| Dalapon | 75-99-0 |

1.2 This is a liquid-solid extraction method and is designed as a simplified alternative to the liquid-liquid extraction approach of Method 552 for the haloacetic acids. This method also provides a much superior technique for the determination of the herbicide, dalapon, compared to the complex herbicide procedure described in Method 515.1. The procedure also represents a major step in the incorporation of pollution prevention in methods development, in that the use of large volumes of organic solvents is eliminated.

1.3 This method is applicable to the determination of the target analytes over the concentration ranges typically found in drinking water (2, 3), subject to the method detection limits (MDL) listed in Table 2. The MDLs observed may vary according to the particular matrix analyzed and the specific instrumentation employed. The haloacetic acids are observed ubiquitously in chlorinated supplies at concentrations ranging from < 1 to > 50 $\mu$g/L.

1.4 Reduced analyte recoveries may be observed in high ionic strength matrices, particularly waters containing elevated sulfate concentrations. Improved recoveries may be obtained by sample dilution at the expense of higher MDLs. This effect is discussed more extensively in Sect. 4.2.

1.5 Tribromoacetic acid has not been included because of problems associated with stability and chromatography with this method.

Mixed bromochloroacetic acids have recently been synthesized. Bromochloroacetic acid is present in chlorinated supplies and method validation data are provided here. Commercial standards are now available for this compound. The mixed trihalogenated acids may also be present. These are not included because of current problems with purity and the chromatography for these compounds.

1.6 This method is designed for analysts skilled in extract concentration techniques, derivatization procedures and the use of GC and interpretation of gas chromatograms.

1.7 When this method is used for the analyses of waters from unfamiliar sources, analyte identifications must be confirmed by at least one additional qualitative technique, such as gas chromatography/mass spectrometry (GC/MS) or by GC using dissimilar columns.

## 2. SUMMARY OF METHOD

2.1 A 100-mL volume of sample is adjusted to pH 5.0 and extracted with a preconditioned miniature anion exchange column. NOTE: The use of liquid-solid extraction disks is certainly permissible as long as all the quality control criteria specified in Sect. 9 of this method are met. The analytes are eluted with small aliquots of acidic methanol and esterified directly in this medium after the addition of a small volume of methyl-tert-butyl ether (MTBE) as co-solvent. The methyl esters are partitioned into the MTBE phase and identified and measured by capillary column gas chromatography using an electron capture detector (GC/ECD).

## 3. DEFINITIONS

3.1 INTERNAL STANDARD (IS) -- A pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same sample or solution. The internal standard must be an analyte that is not a sample component.

3.2 SURROGATE ANALYTE (SA) -- A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction or other processing and is measured with the same procedures used to measure other sample components. The purpose of the SA is to monitor method performance with each sample.

3.3 LABORATORY DUPLICATES (LD1 AND LD2) -- Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 FIELD DUPLICATES (FD1 AND FD2) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.5 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrix that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 FIELD REAGENT BLANK (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.7 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.8 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.9 STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.10 PRIMARY DILUTION STANDARD SOLUTION (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.11 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.12 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentration which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

## 4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from significant interferences under the conditions of the analysis by analyzing laboratory reagent blanks as described in Sect. 9.2.

4.1.1 For each set of samples analyzed, the reagent blank concentration values exceeding 0.1 µg/L should be subtracted from the sample concentrations. A persistent reagent blank of approximately 1 µg/L was observed for bromochloroacetic acid (BCAA) on the primary DB-1701 column. The background was clean on the DB-210 confirmation column and the MDL for BCAA in Table 2 was determined using this column.

4.1.2 Glassware must be scrupulously cleaned (4). Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap water, dilute acid, and reagent water. Drain and heat in an oven or muffle furnace at 400°C for 1 hr. Do not heat volumetric ware. Thermally stable materials such as PCBs may not be eliminated by this treatment. Thorough rinsing with reagent grade acetone may be substituted for the heating. After drying and cooling, store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

4.1.3 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required. The extraction solvent, MTBE, may need to be redistilled.

4.2 The major potential interferences in this ion-exchange procedure are other naturally occurring ions in water sources, principally sulfate. This is the only ion thus far demonstrated as an interference, when present at concentrations possibly occurring in drinking water sources. Sulfate as an effective counter ion displaces the haloacids from the column when present at concentrations above 200 mg/L. Table 4 illustrates this effect for fortified reagent water containing 500 mg/L and 400 mg/L of $Na_2SO_4$ and NaCl respectively (approximately 3.7 millimole (mM) in both cases). Markedly reduced recoveries are observed for all analytes in the presence of high concentrations of sulfate. Reduced recoveries may be observed for the monohaloacetic acids in very high ionic strength waters, as illustrated for the sample with 400 mg/L NaCl. However, normal recoveries were observed from a water sample containing the same molar concentration of $CaCl_2$. The only preventive measure currently available for high ionic strength waters is sample dilution. Dilution by a factor of 5 will suffice in the vast majority of cases, although a factor of 10 may be required in a few extreme sites (e.g. western waters with sulfate > 1000 mg/L). The MDLs will still be approximately 1 µg/L for a dilution factor of 5. However, for many chlorinated supplies the monohaloacetic acids may occur at concentrations near 1 µg/L. In any event, this is the recommended method to determine dalapon.

4.3 The acid forms of the analytes are strong organic acids which react readily with alkaline substances, and can be lost during sample preparation. Glassware must be acid rinsed with 1:9 hydrochloric acid: water prior to use to avoid analyte losses due to adsorption.

4.4 Organic acids and phenols, especially chlorinated compounds, are the most direct potential interferences with the determination. The procedure includes a methanol wash step after the acid analytes are adsorbed on the column. This step eliminates the potential for interferences from neutral or basic, polar organic compounds present in the sample.

4.5 Interfering contamination may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. Routine between-sample rinsing of the sample syringe and associated equipment with MTBE can minimize sample cross-contamination. After analysis of a sample containing high concentrations of analytes, one or more injections of MTBE should be made to ensure that accurate values are obtained for the next sample.

4.6 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the water sampled. Tentative identifications should be confirmed using the confirmation column specified in Table 1 or by the use of gas chromatography with mass spectrometric detection.

## 5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be minimized. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified (5-7) for the information of the analyst.

5.2 The toxicity of the extraction solvent, MTBE, has not been well defined. Susceptible individuals may experience adverse affects upon skin contact or inhalation of vapors. For such individuals a mask may be required. Protective clothing and gloves should be used and MTBE should be used only in a chemical fume hood or glove box.

## 6. EQUIPMENT AND SUPPLIES

6.1 SAMPLE CONTAINERS -- Amber glass bottles, approximately 250 mL, fitted with Teflon-lined screw caps. At least 200 mL of sample should be collected.

6.2 GAS CHROMATOGRAPH (GC) -- Analytical system complete with GC equipped for electron capture detection, split/splitless capillary injection, temperature programming, differential flow control, and with all required accessories including syringes, analytical columns, gases and strip-chart recorder. A data system is recommended for measuring peak areas. The gases flowing through the electron capture detector should be vented through the laboratory fume hood system.

6.3 PRIMARY GC COLUMN -- DB-1701 or equivalent bonded, fused silica column, 30 m x 0.32 mm ID, 0.25 µm film thickness. Another type of column may be used if equivalent or better separation of analytes can be demonstrated.

6.4 CONFIRMATORY GC COLUMN -- DB-210 or equivalent bonded, fused silica column, 30 m x 0.32 mm ID, 0.50 µm film thickness. Another type of column may be used if equivalent or better separation of analytes can be demonstrated.

6.5 PASTEUR PIPETS, GLASS DISPOSABLE

6.6 pH METER -- Wide range with the capability of accurate pH measurements at pH 5 ± 0.5.

6.7 15-mL amber colored bottles with Teflon-lined screw caps.

6.8 LIQUID-SOLID EXTRACTION VACUUM MANIFOLD -- Available from a number of suppliers.

6.9 LSE CARTRIDGES (1 mL) AND FRITS -- Also available from a number of suppliers. The use of LSE disks instead of cartridges is permissible as long as all the quality control criteria in Sect. 9 of this method are met.

6.10 75-mL RESERVOIRS PLUS ADAPTERS -- Available from J. T. Baker, Cat. No. 7120-03 and Cat. No. 7122-00.

6.11 GRADUATED CONICAL CENTRIFUGE TUBES WITH TEFLON-LINED SCREW CAPS (15 mL).

6.12 SCREW CAP CULTURE TUBES -- Suggested size 13 x 100 mm.

6.13 BLOCK HEATER -- Capable of holding screw cap culture tubes in Sect. 6.12.

6.14 VORTEX MIXER

## 7. REAGENTS AND STANDARDS

7.1 REAGENT WATER -- Reagent water is defined as a water in which an interference is not observed at the MDL of each analyte of interest.

7.1.1 A Millipore Super-Q water system or its equivalent may be used to generate deionized reagent water. Distilled water that has been passed through granular charcoal may also be suitable.

7.1.2 Test reagent water each day it is used by analyzing according to Sect. 11.

7.2 METHANOL -- Pesticide quality or equivalent.

7.3 METHYL-TERT-BUTYL ETHER -- Nanograde, redistilled in glass if necessary. Ethers must be demonstrated to be free of peroxides. One test kit (EM Quant Test Strips), is available from EM Science, Gibbstown, NJ. Procedures for removing peroxides from the ether are provided with the test strips. Ethers must be periodically tested (at least monthly) for peroxide formation during use. Any reliable test kit may be used.

7.4 SODIUM SULFATE -- (ACS) granular, anhydrous. Heat in a shallow tray at 400°C for a minimum of 4 hr to remove phthalates and other interfering organic substances. Alternatively, extract with methylene chloride in a Soxhlet apparatus for 48 hr.

7.5 SODIUM HYDROXIDE (NaOH), 1N -- Dissolve 4 g ACS grade in reagent water in a 100-mL volumetric flask and dilute to the line.

7.6 1,2,3-TRICHLOROPROPANE, 99+% -- For use as the internal standard.

7.7 2-BROMOPROPIONIC ACID -- For use as a surrogate compound.

7.8 10% $Na_2SO_4/H_2O$ (BY WEIGHT) SOLUTION -- Dissolve 10g $Na_2SO_4$ in 90 g reagent water.

7.9 10% $H_2SO_4$/MeOH SOLUTION -- Prepare a solution containing 10 mL $H_2SO_4$ in 90 mL methanol.

7.10 1M HCl/MeOH -- Prepare a solution containing 8.25 mL HCl (ACS grade) with 91.75 mL methanol.

7.11 AG-1-X8 ANION EXCHANGE RESIN -- Rinse resin with three consecutive 500-mL aliquots of deionized water and store in deionized water. Available from Biorad, Richmond, CA.

7.12 ACETONE -- ACS reagent grade or equivalent.

7.13 AMMONIUM CHLORIDE -- ACS reagent grade or equivalent.

7.14 SODIUM SULFITE -- ACS reagent grade or equivalent.

7.15 STOCK STANDARD SOLUTIONS

7.15.1 Analytes and Surrogates (Table 1) -- Prepare at 1 to 5 mg/mL in MTBE.

7.15.2 Internal Standard Fortifying Solution -- Prepare a solution of 1,2,3-trichloropropane at 1 mg/mL by adding 36 µL of the neat material (Sect. 7.6) to 50 mL of MTBE. From this stock standard solution, prepare a primary dilution standard at 10 mg/L by the addition of 1 mL to 100 mL MTBE.

7.15.3 Surrogate Standard Fortifying Solution -- Prepare a surrogate stock standard solution of 2-bromopropionic acid at a concentration of 1 mg/mL by accurately weighing approximately 10 mg of 2-bromopropionic acid, transferring it to a 10-mL volumetric, and diluting to the mark with MTBE. Prepare a primary dilution standard at a concentration of 2.5 µg/mL by diluting 250 µL of the stock standard to 100 mL with methanol.

## 8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Grab samples must be collected in accordance with conventional sampling practices (9) using amber glass containers with TFE-lined screw-caps and capacities in excess of 100 mL.

8.1.1 Prior to shipment to the field, to combine residual chlorine, add crystalline ammonium chloride ($NH_4Cl$) to the sample container in an amount to produce a concentration of 100 mg/L in the sample. Alternatively, add 1.0 mL of a 10 mg/mL aqueous solution of $NH_4Cl$ to the sample bottle for each 100 mL of sample bottle capacity immediately prior to sample collection. Granular ammonium chloride may also be added directly to the sample bottle.

8.1.2 After collecting the sample in the bottle containing the dechlorination reagent, seal the bottle and agitate for 1 min.

8.1.3 Samples must be iced or refrigerated at 4°C and maintained at these conditions away from light until extraction. Holding studies performed to date have suggested that, in samples dechlorinated with $NH_4Cl$, the analytes are stable for up to 28 days. Since stability may be matrix dependent, the analyst should verify that the prescribed preservation technique is suitable for the samples under study.

8.1.4 Extract concentrates (Sect. 11.3.6) should be stored at 4°C or less away from light in glass vials with Teflon-lined caps. Extracts should be analyzed within 48 hrs following preparation.

## 9. QUALITY CONTROL

9.1 Minimum quality control (QC) requirements are initial demonstration of laboratory capability, determination of surrogate compound recoveries in each sample and blank, monitoring internal standard peak area or height in each sample and blank, analysis of laboratory reagent blanks, laboratory fortified blanks, laboratory fortified sample matrices, and QC samples. Additional QC practices are recommended.

9.2 LABORATORY REAGENT BLANKS (LRB) -- Before processing any samples, the analyst must analyze at least one LRB to demonstrate that all glassware and reagent interferences are under control. In addition, each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window (Sect. 11.4.4) of any analyte, the LRB produces an interference significantly in excess of that anticipated (Sect. 4.1.1), determine the source of contamination and eliminate the interference before processing samples.

9.3 INITIAL DEMONSTRATION OF CAPABILITY

9.3.1 Select a representative fortified concentration for each of the target analytes. Concentrations near level 2 (Table 4) are recommended. Prepare 4 to 7 replicate laboratory fortified blanks by adding an appropriate aliquot of the primary dilution standard or another certified quality control sample. Be sure to add the internal standard, 1,2,3-trichloropropane, and the surrogate compound, 2 bromopropionic acid, to these samples (See Sect. 11). Analyze the LFBs according to the method beginning in Sect. 11 and calculate mean recoveries and standard deviation for each analyte.

9.3.2 Calculate the mean percent recovery, the standard deviation of the recoveries, and the MDL (10). For each analyte, the mean recovery value, expressed as a percentage of the true value, must fall in the range of 70-130% and the standard deviation should be less than 30%. For those compounds that meet these criteria, performance is considered acceptable and sample analysis may begin. For those compounds that fail these criteria, this procedure must be repeated using a minimum of four fresh samples until satisfactory performance has been demonstrated. Maintain this data on file to demonstrate initial capabilities.

9.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. As laboratory personnel gain experience with this method, the quality of data should improve beyond those required here.

9.3.4 The analyst is permitted to modify GC columns, GC conditions, detectors, extraction techniques, concentration techniques (i.e., evaporation techniques), internal standard or surrogate compounds. Each time such method modifications are made, the analyst must repeat the procedures in Sect. 9.3.1 and also analyze a laboratory fortified matrix sample.

## 9.4 ASSESSING SURROGATE RECOVERY

9.4.1 When surrogate recovery from a sample or blank is < 70% or > 130%, check (1) calculations to locate possible errors, (2) standard solutions for degradation, (3) contamination, and (4) instrument performance. If those steps do not reveal the cause of the problem, reanalyze the extract.

9.4.2 If the extract reanalysis fails the 70-130% recovery criterion, the problem must be identified and corrected before continuing. It may be necessary to extract another aliquot of sample.

9.4.3 If the extract reanalysis meets the surrogate recovery criterion, report only data for the reanalyzed extract. If sample extract continues to fail the recovery criterion, report all data for that sample as suspect.

9.4.4 Develop and maintain control charts on surrogate recovery as described in Sect. 9.6.2. Charting of surrogate recoveries is an especially valuable activity, since these are present in every sample and the analytical results will form a significant record of data quality.

## 9.5 ASSESSING THE INTERNAL STANDARD

9.5.1 When using the internal standard calibration procedure prescribed in this method, the analyst is expected to monitor the IS response (peak area or peak height) of all samples during each analysis day. The IS response for any sample chromatogram should not deviate from the daily calibration standard IS response by more than 30%.

9.5.2 If > 30% deviation occurs with an individual extract, optimize instrument performance and inject a second aliquot of that extract.

9.5.2.1 If the reinjected aliquot produces an acceptable internal standard response, report results for that aliquot.

9.5.2.2 If a deviation of greater than 30% is obtained for the reinjected extract, analysis of the samples should be repeated beginning with Sect. 11, provided the sample is still available. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.

9.5.3 If consecutive samples fail the IS response acceptance criteria, immediately analyze a medium calibration standard.

9.5.3.1 If the calibration standard provides a response factor (RF) within 20% of the predicted value, then follow procedures itemized in Sect. 9.5.2 for each sample failing the IS response criterion.

9.5.3.2 If the check standard provides a response factor which deviates more than 20% of the predicted value, then the analyst must recalibrate (Sect. 10).

## 9.6 LABORATORY FORTIFIED BLANK

9.6.1 The laboratory must analyze at least one laboratory fortified blank (LFB) sample with every 20 samples or one per sample set (all samples extracted within a 24-hr period), whichever is greater. Fortified concentrations near level 2 (Table 4) are recommended. Calculate percent recovery (R). If the recovery of any analyte falls outside the control limits (see Sect. 9.6.2), that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.6.2 Prepare control charts based on mean upper and lower control limits, $R \pm 3 S_R$. The initial demonstration of capability (Sect. 9.3) establishes the initial limits. After each 4-6 new recovery measurements, recalculate R and $S_R$ using all the data, and construct new control limits. When the total number of data points reach 20, update the control limits by calculating R and $S_R$ using only the most recent 20 data points. At least quarterly, replicates of LFBs should be analyzed to determine the precision of the laboratory measurements. Add these results to the ongoing control charts to document data quality.

## 9.7 LABORATORY FORTIFIED SAMPLE MATRIX

9.7.1 Chlorinated water supplies will usually contain significant background concentrations of several method analytes, especially dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA). The concentrations of these acids may be equal to or greater than the fortified concentrations. Table 6 illustrates the relatively poor accuracy and precision which may be anticipated when a large background must be subtracted. The water supply used in the development of this method contained only moderate concentrations of DCAA and TCAA. For many supplies, the concentrations may be so high that fortification may lead to a final extract with instrumental responses exceeding the linear range of the electron capture detector. If this occurs, the extract must be diluted. In spite of these problems, sample sources should be fortified and analyzed as described below. Poor accuracies and high precisions across all analytes likely indicate the presence of interfering ions, especially sulfate, and the requirement for sample dilution.

9.7.2 The laboratory must add known concentrations of analytes to a minimum of 10% of samples or one sample per sample set, whichever is greater. The concentrations should be equal to or greater than the background concentrations in the sample selected for fortification. Ideally, the concentration should be the same as that used for the laboratory fortified blank (Sect. 9.6). Over time, samples from all routine sample sources should be fortified.

9.7.3 Calculate the mean percent recovery, R, of the concentration for each analyte, after correcting the total mean measured concentration, A, from the fortified sample for the background concentration, B, measured in the unfortified sample, i.e.:

$$R = 100 (A - B) / C,$$

where C is the fortifying concentration. Compare these values to control limits appropriate for reagent water data collected in the same fashion (Sect. 9.6).

9.7.4 If the analysis of the unfortified sample reveals the absence of measurable background concentrations, and the added concentrations are those specified in Sect. 9.6, then the appropriate control limits would be the acceptance limits in Sect. 9.6.

9.7.5 If the sample contains measurable background concentrations of analytes, calculate mean recovery of the fortified concentration, R, for each such analyte after correcting for the background concentration (Sect. 9.7.3). Compare these values to reagent water recovery data, R*, at comparable fortified concentrations from Tables 2, 4, and 5. Results are considered comparable if the measured recoveries fall within the range,

$$R \pm 3S_c,$$

where $S_c$ is the estimated percent relative standard deviation in the measurement of the fortified concentration. By contrast to the measurement of recoveries in reagent water (Sect. 9.6.2) or matrix samples without background (Sect. 9.7.3), the relative standard deviation, $S_c$, must be expressed as the statistical sum of variation from two sources, the measurement of the total concentration as well as the measurement of background concentration. In this case, variances, defined as $S^2$, are additive and $S_c$ can be expressed as,

$$S_c^2 = S_a^2 + S_b^2$$

$$\text{or} \quad S_c = (S_a^2 + S_b^2)^{1/2},$$

where $S_a$ and $S_b$ are the percent relative standard deviations of the total measured concentration and the background concentration respectively. The value of $S_a$ may be estimated from the mean measurement of A above or from data at comparable concentrations from Tables 2, 4, and 5. Likewise, $S_b$ can be measured from repetitive measurements of the background concentration or estimated from comparable concentration data from Tables 2, 4, and 5.

9.7.6 If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Sect. 9.6), the recovery problem encountered with the fortified sample is judged to be matrix related, not system related. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.8 QUALITY CONTROL SAMPLE (QCS) -- At least quarterly, analyze a QCS from an external source. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem source.

9.9 The laboratory may adapt additional QC practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage.

## 10. CALIBRATION AND STANDARDIZATION

10.1 Establish GC operating parameters equivalent to the suggested specifications in Table 1. The GC system must be calibrated using the internal standard (IS) technique. Other columns or conditions may be used if equivalent or better performance can be demonstrated.

10.2 INTERNAL STANDARD CALIBRATION PROCEDURE -- This approach requires the analyst to select one or more internal standards which are compatible in analytical behavior with the method analytes. For the single laboratory precision and accuracy data reported in Tables 2-9, one internal standard, 1,2,3-trichloropropane, was used as a concentration of 0.4 µg/mL in the final 5.0-mL concentrate.

10.2.1 Prepare separate stock standard solutions for each analyte of interest at a concentration of 1-5 mg/mL in MTBE. Method analytes may be obtained as neat materials or ampulized solutions (> 99% purity) from a number of commercial suppliers.

10.2.2 Prepare primary dilution standard solutions by combining and diluting stock standard solutions with methanol. As a guideline to the analyst, the primary dilution standard solution used in the validation of this method is described here. Stock standard solutions were prepared in the 1-2 mg/mL range for all analytes and the surrogate. Aliquots of each stock standard solution (approximately 50-250 µL) were added to 100-mL methanol to yield a primary dilution standard containing the following approximate concentrations of analytes:

| | Concentration, µg/mL |
|---|---|
| Monochloroacetic acid | 3 |
| Monobromoacetic acid | 2 |
| Dalapon | 2 |
| Dichloroacetic acid | 3 |
| 2-Bromopropionic acid | 1 |
| Trichloroacetic acid | 1 |
| Bromochloroacetic acid | 2 |
| Dibromoacetic acid | 1 |

The primary dilution standards are used to prepare calibration standards, which comprise at least three concentration levels (optimally five) of each analyte with the lowest standard being at or near the MDL of each analyte. The concentrations of the other standards should define a range containing the expected sample concentrations or the working range of the detector.

10.2.2.1 Calibration standards -- Calibration is performed by extracting procedural standards, i.e.; fortified reagent water. A five-point calibration curve may be prepared by fortifying a 100- mL reagent water samples at pH 5 with 20, 50, 100. 250, and 500 µL of the primary dilution standard prepared above. Alternatively, three levels of calibration solutions may be prepared. Analyze each calibration standard in triplicate according to the procedure outlined in Sect. 11. In addition, a reagent water blank must be analyzed in triplicate.

10.2.3 Include the surrogate analyte, 2-bromopropionic acid, within the calibration standards prepared in Sect. 10.2.2.

10.2.4 Inject 2 µL of each standard and calculate the relative response for each analyte ($RR_a$) using the equation:

$$RR_a = A_a/A_{is}$$

where $A_a$ is the peak area of the analyte.
$A_{is}$ the peak area of the internal standard.

10.2.5 Generate a calibration curve of $RR_a$ versus analyte concentration of the standards expressed in equivalent µg/L in the original aqueous sample. The working calibration curve must be verified daily by measurement of one or more calibration standards. If the response for any analyte falls outside the predicted response by more than 15%, the calibration check must be repeated using a freshly prepared calibration standard. Should the retest fail, a new calibration curve must be generated.

10.2.6 A data system may be used to collect the chromatographic data, calculate response factors, and calculate linear or second order calibration curves.

## 11. PROCEDURE

### 11.1 PREPARATION AND CONDITIONING OF EXTRACTION COLUMNS

11.1.1 Preparation -- Place 1 mL liquid-solid extraction cartridges (Sect. 6.9) onto the vacuum manifold. Place frits into the tubes and push down to place them flat on the bottom. Add the AG-1-X8 resin solution dropwise to the tubes with a Pasteur pipet until there is a solid layer of resin 10 mm in height. Add reagent water and apply vacuum to settle out the suspended resin particles. Do not allow the resin to go dry. At this point extraction of samples can begin or the columns can be stored for later use by maintaining the resin under water and sealing the top with aluminum foil.

11.1.2 Conditioning -- Attach adapters and 75-mL reservoirs to the liquid-solid extraction cartridges. To condition the columns, add to the reservoirs and pass the following series of solvents in 10-mL aliquots through the resin under vacuum: methanol, reagent water, 1 M HCl/MeOH, reagent water, 1 M NaOH, and reagent water. The conditioning solvents should pass through the resin at the rate of ≈ 2 mL/min. without allowing the resin bed to dry and the sample should be added (Sect. 11.2.3) immediately after the last reagent water aliquot.

## 11.2 SAMPLE EXTRACTION AND ELUTION

11.2.1 Remove the samples from storage (Sect. 8.1.3) and allow them to equilibrate to room temperature.

11.2.2 Adjust the pH of a 100-mL sample to 5 ± 0.5 using 1:2 $H_2SO_4$ water and check the pH with a pH meter or narrow range pH paper.

11.2.3 Add 250 µL of the surrogate primary dilution standard (Sect. 7.15.3) to each sample

11.2.4 Transfer the 100-mL sample to the reservoir and apply a vacuum to extract the sample at the rate of ≈ 2 mL/min.

11.2.5 Once the sample has completely passed through the column add 10 mL MeOH to dry the resin.

11.2.6 Remove the reservoirs and adapters, disassemble the vacuum manifold and position screw cap culture tubes (Sect. 6.12) under the columns to be eluted. Reassemble the vacuum manifold, add 4 mL 10% $H_2SO_4$/methanol to the column and elute at the rate of approximately 1.5 mL/min. Turn off the vacuum and remove the culture tubes containing the eluants.

## 11.3 SOLVENT PARTITION

11.3.1 Add 2.5 mL MTBE to each eluant and agitate in the vortex mixer at a low setting for about 5 sec.

11.3.2 Place the capped culture tubes in the heating block (Sect. 6.13) at 50°C and maintain for 1 hr. At this stage, quantitative methylation of all method analytes is attained.

11.3.3 Remove the culture tubes from the heating block and add to each tube 10 mL of 10% by weight of sodium sulfate in reagent water (Sect. 7.8). Agitate each solution for 5-10 sec in the vortex mixer at a high setting.

11.3.4 Allow the phases to separate for approximately 5 min. Transfer the upper MTBE layer to a 15-mL graduated conical centrifuge tube (Sect. 6.11) with a pasteur pipet. Repeat the extraction two more times with approximately 1 mL MTBE each time. Combine the MTBE sample extracts in the graduated centrifuge tube.

11.3.5 Add 200 µL of the internal standard fortifying solution (Sect. 7.15.2) to each extract and add MTBE to each to a final volume of 5 mL.

11.3.6 Transfer a portion of each extract to a vial and analyze using GC-ECD. A duplicate vial should be filled from excess extract. Analyze the samples as soon as possible. The sample extract may be stored up to 48 hr if kept at 4°C or less away from light in glass vials with Teflon-lined caps.

### 11.4 GAS CHROMATOGRAPHY

11.4.1 Table 1 summarizes recommended GC operating conditions and retention times observed using this method. Figure 1 illustrates the performance of the recommended column with the method analytes. Other GC columns, chromatographic conditions, or detectors may be used if the requirements of Sect. 9.3 are met.

11.4.2 Calibrate the system daily as described in Sect. 10. The standards and extracts must be in MTBE.

11.4.3 Inject 2 µL of the sample extract. Record the resulting peak size in area units.

11.4.4 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.4.5 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

## 12. DATA ANALYSIS AND CALCULATIONS

12.1 Calculate analyte concentrations in the sample and reagent blanks from the response for the analyte relative to the internal standard ($RR_a$) using the equation in Sect. 10.2.4.

12.2 For samples processed as part of a set where recoveries falls outside of the control limits established in Sect. 9, results for the affected analytes must be labeled as suspect.

## 13. METHOD PERFORMANCE

13.1 In a single laboratory (EMSL-Cincinnati), recovery and precision data were obtained at three concentrations in reagent water (Tables 2, 4, and 5). In addition, recovery and precision data were obtained at a medium concentration for high ionic strength reagent water (Table 3), dechlorinated tap water, high humectant ground water, and an ozonated surface water (Tables 6-9). The MDL (10) data are given in Table 2.

## 14. POLLUTION PREVENTION

14.1 This method utilizes the new LSE technology which requires the use of very small quantities of organic solvents. This feature eliminates the hazards involved with the use of large volumes of potentially harmful organic solvents needed for conventional liquid-liquid extractions. This method also uses acidic methanol as the derivatizing reagent in place of the highly toxic diazomethane. These features make this method much safer for use by the analyst in the laboratory and much less harmful to the environment.

14.2 For information about pollution prevention that may be applicable to laboratory operations consult "Less is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

## 15. WASTE MANAGEMENT

15.1 Due to the nature of this method there is little need for waste management. No large volumes of solvents or hazardous chemicals are used. The matrices of concern are finished drinking water or source water. However, the Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" also available from the American Chemical Society at the address in Sect. 14.2.

## 16. REFERENCES

1. Hodgeson, J. W., Collins, J. D., and Becker, D. A., "Advanced Techniques for the Measurement of Acidic Herbicides and Disinfection Byproducts in Aqueous Samples," Proceedings of the 14th Annual EPA Conference on Analysis of Pollutants in the Environment, Norfolk, VA., May 8-9, 1991. Office of Water Publication No. 821-R-92-001, U.S. Environmental Protection Agency, Washington, DC, pp 165-194.

2. Uden, P.C. and Miller, J.W., J. Am. Water Works Assoc. 75, 1983, pp. 524-527.

3. Fair. P.S., Barth, R.C., Flesch, J. J. and Brass, H. "Measurement of Disinfection By-products in Chlorinated Drinking Water," Proceedings Water Quality Technology Conference (WQTC-15), Baltimore, Maryland, November 15-20, 1987, American Water Works Association, Denver, CO, pp. 339-353.

4. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.

5. "Carcinogens-Working with Carcinogens," Publication No. 77-206, Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute of Occupational Safety and Health, Atlanta, GA, August 1977.

6. "OSHA Safety and Health Standards, General Industry," (29CFR1910), OSHA 2206, Occupational Safety and Health Administration, Washington, D.C. Revised January 1976.

7. "Safety In Academic Chemistry Laboratories," 3rd Edition, American Chemical Society Publication, Committee on Chemical Safety, Washington, D.C., 1979.

8. Hodgeson, J.W. and Cohen, A.L. and Collins, J.D., "Analytical Methods for Measuring Organic Chlorination By-products," Proceedings Water Quality Technology Conference (WQTC-16), St. Louis, MO, Nov. 13-17, 1988, American Water Works Association, Denver, CO, pp. 981-1001.

9. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.

10. Glaser, J. A., Foerst, D. L., McKee, G. D., Quave, S. A. and Budde, W. L., Environ. Sci. Technol. 15, 1981, pp. 1426-1435.

## 17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

### TABLE 1. RETENTION DATA AND CHROMATOGRAPHIC CONDITIONS

| Analyte | Retention Time, min.<br>Column A | Retention Time, min.<br>Column B |
|---|---|---|
| Monochloroacetic Acid (MCAA) | 5.16 | 9.44 |
| Monobromoacetic Acid (MBAA) | 7.77 | 11.97 |
| Dalapon | 8.15 | 11.97 |
| Dichloroacetic Acid (DCAA) | 8.37 | 11.61 |
| 2-Bromopropionic acid (b) | 8.80 | 12.60 |
| Trichloroacetic Acid (TCAA) | 11.43 | 13.34 |
| 1,2,3-Trichloropropane (a) | 12.62 | 12.91 |
| Bromochloroacetic Acid (BCAA) | 12.92 | 14.20 |
| Dibromoacetic Acid (DBAA) | 15.50 | 16.03 |

Column A: DB-1701, 30 m x 0.32 mm i.d., 0.25 µm film thickness, Injector Temp. = 200°C, Detector Temp. = 260°C, Helium Linear Velocity = 27 cm/sec, Splitless injection with 30 s delay

Program: Hold at 50°C for 10 min, to 200°C at 10°C/min. and hold 5 min., to 230°C at 10°C/min. and hold 5 min.

Column B: DB-210, 30 m x 0.32 mm i.d., 0.50 µm film thickness, Injector Temp. = 200°C, Detector Temp. = 260°C, Linear Helium Flow = 25 cm/sec, splitless injection with 30 s delay.

Program: Hold at 50°C for 10 min., to 200°< at 10°C/min and hold 5 min., to 230° at 10°C/min. and hold 5 min.

(a) Internal Standard

(b) Surrogate Compound

## TABLE 2. ANALYTE RECOVERY AND PRECISION DATA AND METHOD DETECTION LIMITS[a]

LEVEL 1 IN REAGENT WATER

| Analyte | Fortified Conc. µg/L | Mean Meas. Conc. µg/L | Std. Dev. µg/L | Rel. Std. Dev., % | Mean Recovery % | Method Detection Limit µg/L |
|---|---|---|---|---|---|---|
| Monochloroacetic Acid | 1.5 | 1.47 | 0.07 | 4.6 | 98 | 0.21 |
| Monobromoacetic Acid | 1.0 | 0.73 | 0.08 | 7.9 | 73 | 0.24 |
| Dichloroacetic Acid | 1.5 | 1.65 | 0.14 | 7.7 | 110 | 0.45 |
| 2-Bromopropionic Acid[b] | 0.05 | 0.47 | 0.03 | 5.6 | 94 | 0.08 |
| Trichloroacetic Acid | 0.50 | 0.30 | 0.02 | 4.0 | 60 | 0.07 |
| Bromochloroacetic acid | 1.0 | 0.75 | 0.03 | 3.4 | 75 | 0.10 |
| Dibromoacetic Acid | 0.50 | 0.29 | 0.03 | 6.4 | 58 | 0.09 |
| Dalapon | 1.0 | 0.81 | 0.10 | 12 | 81 | 0.32 |

---

[a] Produced by analysis of seven aliquots of fortified reagent water (Reference 10).

[b] Surrogate Compound

## TABLE 3. RECOVERY AND PRECISION DATA IN HIGH IONIC STRENGTH WATERS

MEAN RECOVERY ± RSD[a]

| Analyte | Fortified Conc. µg/L | Reagent Water (RW) | Reagent Water + 500 mg/L $Na_2SO_4$[b] | Reagent Water + 400 mg/L NaCl[b] |
|---|---|---|---|---|
| Monochloroacetic Acid | 7.5 | 109 ± 1.5 | -- | 46 ± 10 |
| Monobromoacetic Acid | 5.0 | 83 ± 18 | 5.0 ± 10 | 50 ± 13 |
| Dichloroacetic Acid | 7.5 | 107 ± 3.6 | 59 ± 2.4 | 114 ± 0.1 |
| 2-Bromopropionic Acid | 2.5 | 108 ± 1.8 | 32 ± 0.3 | 137 ± 2.1 |
| Trichloroacetic Acid | 2.5 | 101 ± 0.4 | 8 ± 3.0 | 64 ± 11 |
| Bromochloroacetic Acid | 5.0 | 101 ± 2.6 | 85 ± 0.7 | 107 ± 3.5 |
| Dibromoacetic Acid | 2.5 | 93 ± 1.9 | 40 ± 22 | 89 ± 5.0 |
| Dalapon | 5.0 | 93 ± 1.9 | 57 ± 5.3 | 99 ± 1.7 |

[a] Based on the analysis of three replicate samples.

[b] Molar concentration of added salt is 3.7 mM in both cases.

## TABLE 4. ANALYTE RECOVERY AND PRECISION DATA[a]

LEVEL 2 IN REAGENT WATER

| Analyte | Fortified Conc. µg/L | Mean Meas. Conc. µg/L | Std. Dev. µg/L | Rel. Std. Dev., % | Mean Recovery % |
|---|---|---|---|---|---|
| Monochloroacetic Acid | 7.5 | 7.73 | 0.18 | 2.3 | 103 |
| Monobromoacetic Acid | 5.0 | 3.95 | 0.65 | 16 | 79 |
| Dichloroacetic Acid | 7.5 | 8.06 | 0.16 | 2.0 | 108 |
| 2-Bromopropionic Acid[b] | 2.5 | 2.57 | 0.06 | 2.4 | 103 |
| Trichloroacetic Acid | 2.5 | 2.32 | 0.14 | 5.8 | 93 |
| Bromochloroacetic Acid | 5.0 | 5.22 | 0.12 | 2.2 | 104 |
| Dibromoacetic Acid | 2.5 | 2.41 | 0.09 | 3.4 | 96 |
| Dalapon | 5.0 | 4.03 | 0.36 | 7.5 | 97 |

[a] Produced by the analysis of seven aliquots of fortified reagent water.

[b] Surrogate Compound

## TABLE 5. ANALYTE RECOVERY AND PRECISION DATA[a]

### LEVEL 3 IN REAGENT WATER

| Analyte | Fortified Conc. µg/L | Mean Meas. Conc. µg/L | Std. Dev. µg/L | Rel. Std. Dev., % | Mean Recovery % |
|---|---|---|---|---|---|
| Monochloroacetic Acid | 15.0 | 14.5 | 0.15 | 1.0 | 99 |
| Monobromoacetic Acid | 10.0 | 7.82 | 0.68 | 8.4 | 78 |
| Dichloroacetic Acid | 15.0 | 15.1 | 6.09 | 0.6 | 101 |
| 2-Bromopropionic Acid | 5.0 | 4.98 | 0.08 | 1.5 | 100 |
| Trichloroacetic Acid | 5.0 | 4.89 | 0.07 | 1.4 | 98 |
| Bromochloroacetic Acid | 10.0 | 10.3 | 0.25 | 2.4 | 103 |
| Dibromoacetic Acid | 5.0 | 4.85 | 0.04 | 0.7 | 97 |
| Dalapon | 10.0 | 9.02 | 0.16 | 1.8 | 90 |

---

[a] Produced by the analysis of seven aliquots of fortified reagent water.

## TABLE 6. ANALYTE RECOVERY AND PRECISION DATA[a]

DECHLORINATED TAP WATER

| Analyte | Fortified Conc. µg/L | Mean[b] Meas. Conc. µg/L | Std. Dev. µg/L | Rel. Std. Dev., % | Mean Recovery % |
|---|---|---|---|---|---|
| Monochloroacetic Acid | 7.5 | 5.70 | 0.63 | 11 | 76 |
| Monobromoacetic Acid | 5.0 | 4.57 | 0.45 | 9.8 | 91 |
| Dichloroacetic Acid | 7.5 | 5.62 | 0.76 | 14 | 75 |
| 2-Bromopropionic Acid[c] | 7.5 | 2.22 | 0.16 | 7.2 | 89 |
| Trichloroacetic Acid | 2.5 | 1.48 | 0.42 | 28 | 59 |
| Bromochloroacetic Acid | 5.0 | 5.70 | 0.92 | 16 | 114 |
| Dibromoacetic Acid | 2.5 | 2.42 | 0.13 | 5.4 | 97 |
| Dalapon | 5.0 | 4.69 | 0.21 | 4.5 | 94 |

---

[a] Produced by the analysis of seven aliquots of fortified dechlorinated tap water.

[b] Significant background concentrations (> 5-15 µg/L) have been subtracted from these values for dichloroacetic acid, trichloroacetic acid, bromochloroacetic acid, and dibromoacetic acid.

[c] Surrogate Compound

## TABLE 7. ANALYTE RECOVERY AND PRECISION DATA[a]

### HIGH HUMIC CONTENT GROUND WATER

| Analyte | Fortified Conc. µg/L | Mean Meas. Conc. µg/L | Std. Dev. µg/L | Rel. Std. Dev., % | Mean Recovery % |
|---|---|---|---|---|---|
| Monochloroacetic Acid | 7.5 | 3.55 | 0.32 | 8.9 | 47 |
| Monobromoacetic Acid | 5.0 | 2.21 | 0.21 | 11 | 44 |
| Dichloroacetic Acid | 7.5 | 7.60 | 0.08 | 1.1 | 101 |
| 2-Bromopropionic Acid[b] | 2.5 | 1.83 | 0.09 | 4.9 | 73 |
| Trichloroacetic Acid | 2.5 | 2.37 | 0.12 | 5.1 | 95 |
| Bromochloroacetic Acid | 5.0 | 5.53 | 0.16 | 2.9 | 111 |
| Dibromoacetic Acid | 2.5 | 2.58 | 0.13 | 5.0 | 103 |
| Dalapon | 5.0 | 4.92 | 0.29 | 6.0 | 90 |

[a] Produced by the analysis of seven aliquots of fortified high humic content ground water.

[b] Surrogate Compound

## TABLE 8. ANALYTE RECOVERY AND PRECISION DATA[a]

HIGH HUMIC CONTENT GROUND WATER DILUTED 1:5

| Analyte | Fortified Conc. µg/L | Mean Meas. Conc. µg/L | Std. Dev. µg/L | Rel. Std. Dev., % | Mean Recovery % |
|---|---|---|---|---|---|
| Monochloroacetic Acid | 1.5 | 1.50 | 0.17 | 11 | 100 |
| Monobromoacetic Acid | 1.0 | 0.97 | 0.06 | 6.2 | 97 |
| Dichloroacetic Acid | 1.5 | 1.89 | 0.16 | 8.5 | 126 |
| 2-Bromopropionic Acid[b] | 0.5 | 0.49 | 0.01 | 2.0 | 98 |
| Trichloroacetic Acid | 0.5 | 0.28 | 0.03 | 11 | 56 |
| Bromochloroacetic Acid | 1.0 | 0.43 | 0.07 | 16 | 43 |
| Dibromoacetic Acid | 0.5 | 0.30 | 0.02 | 6.7 | 60 |
| Dalapon | 1.0 | 0.88 | 0.12 | 14 | 88 |

[a] Produced by the analysis of seven aliquots of fortified high humic content ground water diluted 1:5.

[b] Surrogate Compound

## TABLE 9. ANALYTE RECOVERY AND PRECISION DATA[a]

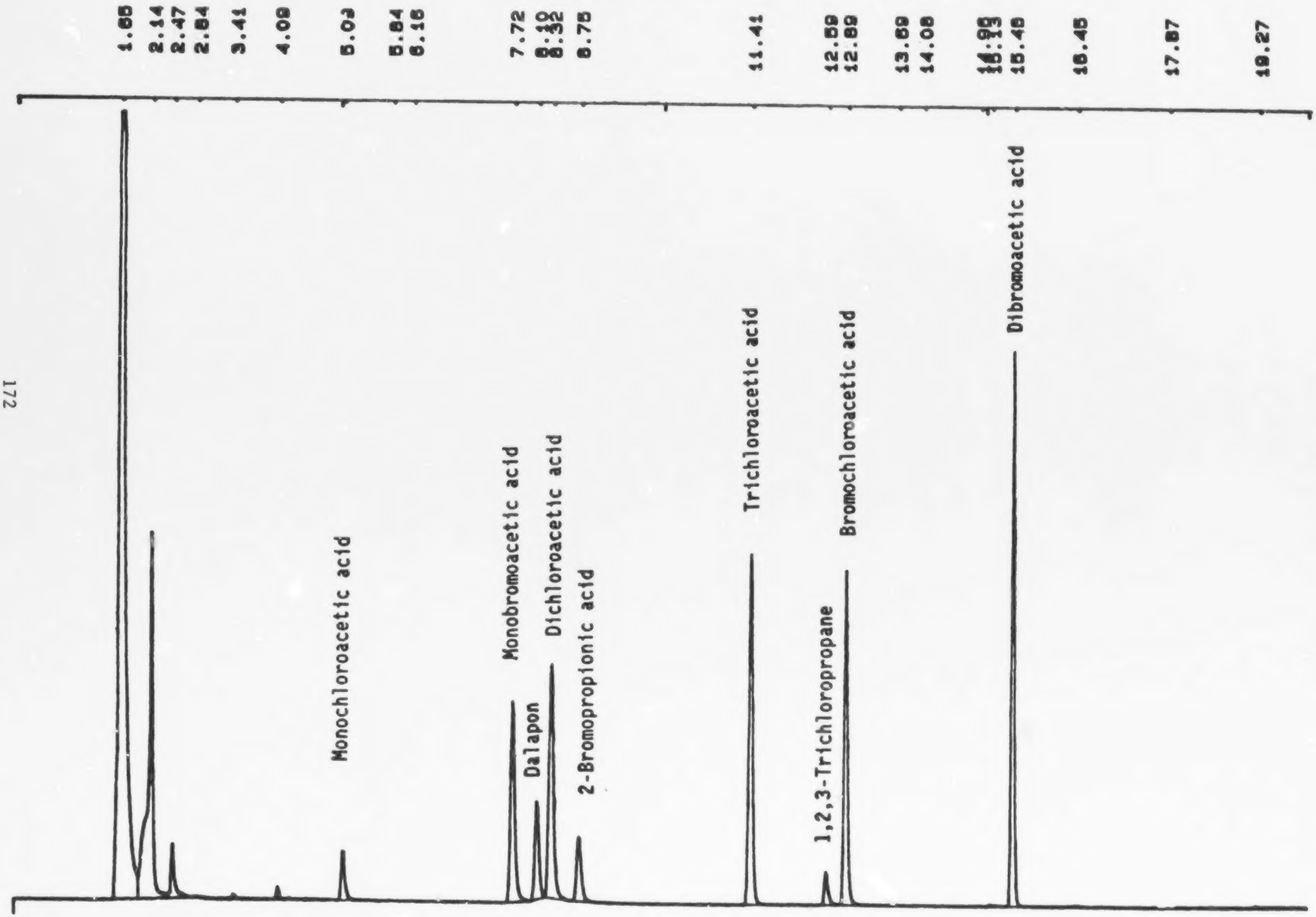
OZONATED RIVER WATER

| Analyte | Fortified Conc. µg/L | Mean Meas. Conc. µg/L | Std. Dev. µg/L | Rel. Std. Dev., % | Mean Recovery % |
|---|---|---|---|---|---|
| Monochloroacetic Acid | 7.5 | 6.22 | 0.91 | 15 | 83 |
| Monobromoacetic Acid | 5.0 | 4.28 | 0.34 | 7.9 | 86 |
| Dichloroacetic Acid | 7.5 | 7.09 | 0.22 | 3.1 | 94 |
| 2-Bromopropionic Acid[b] | 2.5 | 2.31 | 0.09 | 3.7 | 92 |
| Trichloroacetic Acid | 2.5 | 2.65 | 0.13 | 4.9 | 106 |
| Bromochloroacetic Acid | 5.0 | 5.20 | 0.18 | 3.5 | 104 |
| Dibromoacetic Acid | 2.5 | 2.36 | 0.09 | 3.8 | 94 |
| Dalapon | 5.0 | 5.08 | 0.17 | 3.4 | 102 |

[a] Produced by the analysis of seven aliquots of fortified ozonated river water.

[b] Surrogate Compound

FIGURE 1. Gas Chromatogram of Haloacetic Acids Including the Internal Standard and Surrogate Compound Using the DB-1701 Column and the Conditions Listed in Table 1



# METHOD 553. DETERMINATION OF BENZIDINES AND NITROGEN-CONTAINING PESTICIDES IN WATER BY LIQUID-LIQUID EXTRACTION OR LIQUID-SOLID EXTRACTION AND REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/ PARTICLE BEAM/MASS SPECTROMETRY

Revision 1.1

August 1992

Thomas D. Behymer
Thomas A. Bellar
James S. Ho
William L. Budde

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

## METHOD 553

## DETERMINATION OF BENZIDINES AND NITROGEN-CONTAINING PESTICIDES IN WATER BY LIQUID-LIQUID EXTRACTION OR LIQUID-SOLID EXTRACTION AND REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/PARTICLE BEAM/MASS SPECTROMETRY

### 1. SCOPE AND APPLICATION

1.1 This is a general purpose method that provides procedures for determination of benzidines and nitrogen-containing pesticides in water and wastewater. The method is applicable to a wide range of compounds that are efficiently partitioned from a water sample into methylene chloride or onto a liquid-solid extraction device. The compounds must also be amenable to separation on a reverse phase liquid chromatography column and transferable to the mass spectrometer with a particle beam interface. Particulate bound organic matter will not be partitioned onto the liquid-solid extraction system, and more than trace levels of particulates in the water may disrupt the partitioning process. The compounds listed below are potential method analytes and single-laboratory accuracy and precision data have been determined for the compounds as described in Sect. 13. The specific analytical conditions given in the method are applicable to those compounds for which accuracy and precision data are given. Other analytes (Sect. 1.2) may require slight adjustments of analytical conditions. A laboratory may use this method to identify and measure additional analytes after the laboratory obtains acceptable (defined in Sect. 9) accuracy and precision data for each added analyte.

| Compound | Abbreviation | MW[1] | Chemical Abstracts Service Registry Number (CASRN) |
|---|---|---|---|
| benzidine | BZ | 184 | 92-87-5 |
| benzoylprop ethyl | BP | 365 | 33878-50-1 |
| caffeine | CF | 194 | 58-08-2 |
| carbaryl | CL | 201 | 63-25-2 |
| o-chlorophenyl thiourea | PT | 186 | 5344-82-1 |
| 3,3'-dichlorobenzidine | DB | 252 | 91-94-1 |
| 3,3'-dimethoxybenzidine | MB | 244 | 119-90-4 |
| 3,3'-dimethylbenzidine | LB | 212 | 612-82-8 |
| diuron | DI | 232 | 330-54-1 |
| ethylene thiourea | ET | 102 | 96-45-7 |
| linuron (Lorox) | LI | 248 | 330-55-2 |
| monuron | MO | 198 | 150-68-5 |
| rotenone | RO | 394 | 83-79-4 |
| siduron | SI | 232 | 1982-49-6 |

[1] Monoisotopic molecular weight calculated from the atomic masses of the isotopes with the smallest masses.

1.2 Preliminary investigation indicates that the following compounds may be amenable to this method: Aldicarb sulfone, Carbofuran, Methiocarb, Methomyl (Lannate), Mexacarbate (Zectran), and N-(1-Naphthyl) thiourea. Caffeine, Ethylene thiourea and o-Chlorophenyl thiourea have been successfully analyzed by HPLC/PB/MS, but have not been successfully extracted from a water matrix.

1.3 Method detection limit (MDL) is defined as the statistically calculated minimum amount that can be measured with 99% confidence that the reported value is greater than zero (1). The MDL is compound dependent and is particularly dependent on extraction efficiency and sample matrix. For the analytes listed in Tables 3-5, the estimated MDLs range from 2 to 30 $\mu$g/L.

## 2. SUMMARY OF METHOD

2.1 Organic compound analytes and surrogates are extracted from 1 L of water sample by liquid-liquid extraction (LLE) with methylene chloride or by passing 1 L of sample water through a cartridge or disk containing a solid inorganic matrix coated with a chemically bonded $C_{18}$ organic phase or a neutral polystyrene/divinylbenzene polymer (liquid-solid extraction, LSE). If LLE is used, the analytes are concentrated in methanol by evaporation of the methylene chloride and addition of methanol (solvent exchange). If LSE is used, the analytes are eluted from the LSE cartridge or disk with a small quantity of methanol and concentrated further by evaporation of some of the solvent. The sample components are separated, identified, and measured by injecting an aliquot of the concentrated methanol solution into a high performance liquid chromatograph (HPLC) containing a reverse phase HPLC column and interfaced to a mass spectrometer (MS) with a particle beam (PB) interface. Compounds eluting from the HPLC column are identified by comparing their measured mass spectra and retention times to reference spectra and retention times in a data base. Reference spectra and retention times for analytes are obtained by measurement of calibration standards under the same conditions used for samples. The concentration of each identified component is measured by relating the MS response of the quantitation ion produced by that compound to the MS response of the quantitation ion produced by the same compound in a calibration standard (external standard). Surrogate analytes, whose concentrations are known in every sample, are measured with the same external standard calibration procedure. An optional isotope dilution procedure is included for samples which contain interfering matrix or coeluting compounds.

## 3. DEFINITIONS

3.1 EXTERNAL STANDARD (ES) -- A pure analyte(s) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard(s) is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the sample.

3.2 SURROGATE ANALYTE (SA) -- A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction and is measured with the same procedures used to measure other sample components. The purpose of a surrogate analyte is to monitor method performance with each sample.

3.3 LABORATORY DUPLICATES (LD1 and LD2) -- Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 FIELD DUPLICATES (FD1 and FD2) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.5 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 FIELD REAGENT BLANK (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.7 INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

3.8 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.9 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.10 STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.11 PRIMARY DILUTION STANDARD SOLUTION (PDS)-- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.12 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.13 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.14 INSTRUMENT DETECTION LIMIT (IDL) -- The minimum quantity of analyte or the concentration equivalent which gives an analyte signal equal to three times the standard deviation of the background signal at the selected wavelength, mass, retention time, absorbance line, etc.

## 4. INTERFERENCES

4.1 When two compounds coelute, the transport efficiency of both compounds through the particle beam interface generally improves and enhanced ion abundances are observed in the mass spectrometer (2). The degree of signal enhancement by coelution is compound dependent. This coelution effect invalidates the external calibration curve and, if not recognized, will result in incorrect concentration measurements. Procedures given in this method to check for coeluting compounds must be followed to preclude inaccurate measurements (Sect. 10.2.6.5 and Sect. 12.1). An optional isotope dilution calibration procedure has been included for use when interfering matrix or coeluting compounds are present.

4.2 During analysis, major contaminant sources are reagents, chromatography columns, and liquid-solid extraction columns or disks.

Analyses of field and laboratory reagent blanks provide information about the presence of contaminants.

4.3 Interfering contamination may occur when a sample containing low concentrations of compounds is analyzed immediately after a sample containing relatively high concentrations of compounds. Syringes, injectors, and other equipment must be cleaned carefully or replaced as needed. After analysis of a sample containing high concentrations of compounds, a laboratory reagent blank should be analyzed to ensure that accurate values are obtained for the next sample.

## 5. SAFETY

5.1 The toxicity or carcinogenicity of chemicals used in this method has not been precisely defined; each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining awareness of procedures and regulations for safe handling of chemicals used in this method (3-5).

5.2 Some method analytes have been tentatively classified as known or suspected human or mammalian carcinogens. Pure standard materials and stock standard solutions of all analytes should be handled with suitable protection to skin, eyes, etc.

## 6. EQUIPMENT AND SUPPLIES

6.1 All glassware must be meticulously cleaned. This may be accomplished by washing with detergent and water, rinsing with water, distilled water, or solvents, air-drying, and heating (where appropriate) in an oven. Volumetric glassware is never heated.

6.2 SAMPLE CONTAINERS -- 1-L or 1-qt amber glass bottles fitted with a Teflon-lined screw cap. (Bottles in which high purity solvents were received can be used as sample containers without additional cleaning if they have been handled carefully to avoid contamination during use and after use of original contents.)

6.3 SEPARATORY FUNNELS -- 2-L and 100-mL with a Teflon stopcock.

6.4 LIQUID CHROMATOGRAPHY COLUMN RESERVOIRS -- Pear-shaped 100- or 125-mL vessels without a stopcock but with a ground glass outlet sized to fit the liquid-solid extraction column. (Lab Glass, Inc., Part No. ML-700-706S, with a 24/40 top outer joint and a 14/35 bottom inner joint, or equivalent.) A 14/35 outlet joint fits some commercial cartridges.

6.5 SYRINGE NEEDLES -- No. 18 or 20 stainless steel.

6.6 VACUUM FLASKS -- 1 or 2 L with solid rubber stoppers.

6.7 VOLUMETRIC FLASKS -- Various sizes.

6.8 LABORATORY OR ASPIRATOR VACUUM SYSTEM -- Sufficient capacity to maintain a slight vacuum of 13 cm (5 in) of mercury in the vacuum flask.

6.9 MICRO SYRINGES -- Various sizes.

6.10 VIALS -- Various sizes of amber vials with Teflon-lined screw caps.

6.11 DRYING COLUMN -- 0.6 cm x 40 cm with 10 mL graduated collection vial.

6.12 CONCENTRATOR TUBE -- Kuderna-Danish (K-D) 10 mL graduated with ground glass stoppers.

6.13 ANALYTICAL BALANCE -- Capable of weighing 0.0001 g accurately.

6.14 LIQUID CHROMATOGRAPHY COLUMN -- A 15-25 cm x 2 mm (i.d.) stainless steel tube (e.g., Waters C-18 Novapak or equivalent) packed with silica particles (4-10 μm) with octadecyldimethylsilyl (C-18) groups chemically bonded to the silica surface. Residual acidic sites should be blocked (endcapped) with methyl or other non-polar groups and the stationary phase must be bonded to the solid support to minimize column bleed. Column selection for minimum bleeding is strongly recommended. The column must be conditioned over night before each use by pumping a 75-100% v/v acetonitrile: water solution through it at a rate of about 0.05 mL/min. Other packings and column sizes may be used if equivalent or better performance can be achieved.

6.15 Guard column of similar packing used in the analytical column is recommended.

6.16 LIQUID CHROMATOGRAPH/MASS SPECTROMETER/DATA SYSTEM (LC/MS/DS)

6.16.1 The LC must accurately maintain flow rates between 0.20-0.40 mL/min while performing a gradient elution from 100% solvent A to 100% solvent B. Pulse dampening is recommended but not required. An autoinjector is highly desirable and should be capable of accurately delivering 1-10 μL injections without affecting the chromatography.

6.16.2 The system should include a post-column mixing tee and an additional LC pump for post-column addition of acetonitrile at a constant rate of 0.1 - 0.7 mL/min.

6.16.3 The particle beam LC/MS interface must reduce the system pressure to a level fully compatible with the generation of classical electron ionization (EI) mass spectra, i.e., about $1 \times 10^{-6}$ to $1 \times 10^{-4}$ Torr, while delivering sufficient quantities of analytes to the conventional EI source to meet sensitivity, accuracy, and precision requirements.

All significant background components with mass greater than 62 Daltons should be removed to a level that does not produce ions greater than a relative abundance of 10% in the mass spectra of the analytes.

6.16.4 The mass spectrometer must be capable of electron ionization at a nominal electron energy of 70 eV. The spectrometer must be capable of scanning from 45 to 500 amu with a complete scan cycle time (including scan overhead) of 1.5 sec or less. (Scan cycle time = Total MS data acquisition time in seconds divided by number of scans in the chromatogram). The spectrometer must produce a mass spectrum that meets all criteria in Table 1 when 500 ng or less of DFTPPO (Sect. 7.11) is introduced into the LC. An average spectrum across the DFTPPO LC peak may be used to test instrument performance.

6.16.5 An interfaced data system is required to acquire, store, reduce, and output mass spectral data. The computer software should have the capability of processing stored LC/MS data by integration of the ion abundance of any specific ion between specified time or scan number limits, construction of a first or second order regression calibration curves, calculation of response factors as defined in Sect. 10.2.9, calculation of response factor statistics (mean and standard deviation), and calculation of concentrations of analytes from the calibration curve or the equation in Sect. 12.

6.17 MILLIPORE STANDARD FILTER APPARATUS, ALL GLASS -- This will be used if the disks are to be used to carry out the extraction instead of cartridges.

## 7. REAGENTS AND STANDARDS

7.1 Helium nebulizer/carrier gas as contaminant free as possible.

7.2 LIQUID-SOLID EXTRACTION (LSE) MATERIALS

7.2.1 Cartridges are inert non-leaching plastic, for example polypropylene, or glass and must not contain contaminants that leach into methanol. The cartridges are packed with various amounts of sorbents such as $C_{18}$ or a neutral polystyrene/divinylbenzene polymer. The packing must have a narrow size distribution and must not leach organic compounds into methanol. One liter of water should pass through the cartridge in about 2 hr with the assistance of a slight vacuum of about 13 cm (5 in) of mercury. Faster flow rates are acceptable if equivalent accuracy and precision are obtained. Robotic systems typically pump the sample through a cartridge in less than 2 hr. These systems are also acceptable if equivalent accuracy and precision are obtained. Sect. 9 and Tables 4 and 5 provide criteria for acceptable LSE cartridges which are available from several commercial suppliers.

7.2.2 Extraction disks (Empore) are thin filter-shaped materials with $C_{18}$ modified silica, or neutral polystyrene/divinylbenzene polymer, impregnated in a Teflon or other inert matrix. As with cartridges, the disks should not contain any organic compounds, either from the Teflon or the bonded silica, which will leach into the methanol eluant. One liter of reagent water should pass through the disks in 5-20 min using a vacuum of about 66 cm (26 in) of mercury. Sect. 9 provides criteria for acceptable LSE disks which are available commercially.

7.3 SOLVENTS

7.3.1 Acetonitrile, methylene chloride, and methanol -- HPLC grade and pesticide quality or equivalent.

7.3.2 Reagent water -- Water in which an interferant is not observed at the MDL of the compound of interest. Prepare reagent water by passing tap water through a filter bed containing about 0.5 kg of activated carbon or by using a water purification system. Store in clean, narrow-mouth bottles with Teflon-lined septa and screw caps.

7.4 Hydrochloric acid, concentrated.

7.5 Sodium sulfate, anhydrous.

7.6 REDUCING AGENTS FOR CHLORINATED WATER -- Sodium sulfite, sodium thiosulfate or sodium arsenite.

7.7 AMMONIUM ACETATE, SODIUM CHLORIDE, AND SODIUM HYDROXIDE (1N) -- ACS reagent grade.

7.8 STOCK STANDARD SOLUTIONS (SSS) -- Individual solutions of analytes, surrogates, and isotopically labelled analogues of the analytes may be purchased as certified solutions or prepared from pure materials. To prepare, add 10 mg (weighed on an analytical balance to 0.1 mg) of the pure material to 1.9 mL of methanol or acetonitrile in a 2-mL volumetric flask, dilute to the mark, and transfer the solution to an amber glass vial. Certain analytes, such as 3,3'-dimethoxybenzidine, may require dilution in 50% v/v acetonitrile or methanol: water solution. If the analytical standard is available only in quantities smaller than 10 mg, reduce the volume of solvent accordingly. If compound purity is certified by the supplier at >96%, the weighed amount can be used without correction to calculate the concentration of the solution (5 μg/μL). Store the amber vials in a freezer at < 0°C.

7.8.1 Benzidines as the free base or as acid chlorides may be used for calibration purposes. However, the concentration of the standard must be calculated as the free base.

7.9 PRIMARY DILUTION STANDARD SOLUTION (PDS) -- The stock standard solutions are used to prepare a primary dilution standard solution that contains multiple analytes. The recommended solvent for this dilution is a 50% v/v acetonitrile:water mixture. Aliquots of each of the stock standard solutions are combined to produce the primary dilution in which the concentration of the analytes is at least equal to the concentration of the most concentrated calibration solution. Store the primary dilution standard solution in an amber vial in a freezer at < 0°C, and check frequently for signs of deterioration or evaporation, especially just before preparing calibration solutions.

7.10 FORTIFICATION SOLUTION OF SURROGATES -- The analyst should monitor the performance of the extraction, cleanup (when used), and analytical system and the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard, and blank with 1 or 2 surrogates recommended to encompass the range of the gradient elution program used in this method. The compounds recommended as surrogates for the analysis of benzidines and nitrogen-containing pesticides are benzidine-$D_8$ (DBZ), caffeine-$^{15}N_2$ (NCF), 3,3'-dichlorobenzidine-$D_6$ (DCB), and bis-(perfluorophenyl)-phenylphosphine oxide (OD) unless their unlabelled counterpart is being analyzed or they will be used for isotope dilution calibration (Abbreviations in parentheses are used in Figure 4). Prepare a solution of the surrogates in methanol or acetonitrile at a concentration of 5 mg/mL of each. Other surrogates may be included in this solution as needed. (A 10-µL aliquot of this solution added to 1 L of water gives a concentration of 50 µg/L of each surrogate). Store the surrogate fortifying solution in an amber vial in a freezer at < 0°C.

7.11 MS PERFORMANCE CHECK SOLUTION -- Prepare a 100 ng/µL solution of bis-(perfluorophenyl)-phenylphosphine oxide (DFTPPO) in acetonitrile. Store this solution in an amber vial in a freezer at < 0°C. DFTPPO is not currently commercially available. For this method development work, DFTPPO was synthesized from bis-(perfluorophenyl) phenyl phosphine (DFTPP) in solution by adding a slight excess of hydrogen peroxide (DFTPP + $H_2O_2$ → DFTPPO + $H_2O$). The solvent was removed and the resulting crystals were thoroughly washed with water to remove any residual hydrogen peroxide. It is critical to remove all residual hydrogen peroxide before adding the DFTPPO to the CAL solution. Any residual hydrogen peroxide will degrade some analytes.

7.12 CALIBRATION SOLUTIONS (CAL1 - CAL6) -- Prepare a series of six concentration calibration solutions in acetonitrile which contain all analytes at concentrations of 2, 5, 10, 25, 50 and 100 times the instrument detection limit of each compound with a constant concentration of each surrogate in each CAL solution. This calibration range may be optimized by the operator, but each analyte must be bracketed by at least 2 calibration points. CAL1 through CAL6 are prepared by combining appropriate aliquots of the primary dilution standard solution (Sect. 7.9) and the fortification solution of surrogates (Sect. 7.10). DFTPPO may be added to one or more CAL solutions to verify MS tune (See Sect. 10.3.1.). Store these solutions in amber vials in a freezer at < 0°C. Check these solutions quarterly for signs of deterioration.

7.12.1 For isotope dilution calibration, prepare the calibration solutions as described above with the addition of one coeluting isotopically labelled analog for each analyte of interest. The concentration for each coeluting labelled standard should be approximately 25 to 50 times the instrument detection limit of the analyte of interest and must be constant in all calibration solutions (CAL1 through CAL6). These solutions permit the relative response (unlabelled to labelled) to be measured as a function of the amount of analyte injected. If more than one labelled compound is used, one spiking solution containing all labelled compounds should be prepared.

7.13 MOBILE PHASE -- Solvent A is a 75:25 v/v water:acetonitrile solution containing ammonium acetate at a concentration of 0.01 M. This composition is used to eliminate biological activity in the A Phase. Solvent B is acetonitrile. Both solvents are degassed in an ultrasonic bath under reduced pressure and maintained by purging with a low flow of helium.

## 8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE COLLECTION -- When sampling from a water tap, open the tap and allow the system to flush until the water temperature has stabilized (usually about 2-5 min). Adjust the flow to about 500 mL/min and collect samples from the flowing stream. Keep samples sealed from collection time until analysis. When sampling from an open body of water, fill the sample container with water from a representative area. Sampling equipment, including automatic samplers, must be free of plastic tubing, gaskets, and other parts that may leach analytes into water. Automatic samplers that composite samples over time must use refrigerated glass sample containers.

8.2 SAMPLE DECHLORINATION AND PRESERVATION -- All samples should be iced or refrigerated at 4°C from the time of collection until extraction. Residual chlorine should be reduced at the sampling site by addition of a reducing agent. Add 40-50 mg of sodium sulfite or sodium thiosulfate (these may be added as solids with stirring until dissolved) to each liter of water.

8.3 HOLDING TIME -- Samples must be extracted within 7 days and the extracts analyzed within 30 days of sample collection. Extracts should be stored in an amber vial in a freezer at < 0°C.

8.4 FIELD BLANKS

8.4.1 Processing of a field reagent blank (FRB) is recommended along with each sample set, which is composed of the samples collected from the same general sample site at approximately the same time. At the laboratory, fill a sample container with reagent water, seal, and ship to the sampling site along with the empty sample containers. Return the FRB to the laboratory with filled sample bottles.

## 9. QUALITY CONTROL

9.1 Quality control (QC) requirements are the initial demonstration of laboratory capability followed by regular analyses of LRBs, LFBs, and laboratory fortified matrix samples. The laboratory must maintain records to document the quality of the data generated. Additional QC practices are recommended.

9.2 Initial demonstration of low system background and acceptable particle size and packing. Before any samples are analyzed, or any time a new supply of LSE cartridges or disks is received from a supplier, or a new column is installed, it must be demonstrated that a LRB is reasonably free of contamination that would prevent the determination of any analyte of concern. In this same experiment it must be demonstrated that the particle size and packing of the LSE cartridge are acceptable. Consistent flow rate is an indication of acceptable particle size distribution and packing.

9.2.1 A source of potential contamination may be the liquid-solid extraction (LSE) cartridges and disks and columns which may contain silicon compounds and other contaminants that could prevent the determination of method analytes. Generally, contaminants will be leached from the cartridges, disks, or columns into the solvent and produce a variable background. If the background contamination is sufficient to prevent accurate and precise analyses, this condition must be corrected before proceeding with the initial demonstration. Figure 1 shows unacceptable background contamination from a column with stationary phase bleed.

9.2.2 Other sources of background contamination are solvents, reagents, and glassware. Background contamination must be reduced to an acceptable level before proceeding with the next section. In general, background for method analytes should be below the MDL.

9.2.3 One liter of water should pass through the cartridge in about 2 hr (faster flow rates are acceptable if precision and accuracy are acceptable) with a partial vacuum of about 13 cm (5 in) of mercury. The extraction time should not vary unreasonably among LSE cartridges. Robotic systems typically pump the sample through a cartridge in less than 2 hr. These systems are also acceptable if equivalent accuracy and precision are obtained. Extraction disks may be used at a faster flow rate (See Sect. 7.2.2).

9.3 INITIAL DEMONSTRATION OF LABORATORY ACCURACY AND PRECISION -- Analyze 5-7 replicates of a LFB containing each analyte of concern at a concentration in the range of 10-50 times the instrument detection limits (see regulations and maximum contaminant levels for guidance on appropriate concentrations).

9.3.1 Prepare each replicate by adding an appropriate aliquot of the PDS, or another certified quality control sample, to reagent water. Analyze each replicate according to the procedures described in Sect. 11 and on a schedule that results in the analyses of all replicates with 48 hr.

9.3.2 Calculate the measured concentration of each analyte in each replicate, the mean concentration of each analyte in all replicates, and mean accuracy (as mean percentage of true value) for each analyte, and the precision (as relative standard deviation, RSD) of the measurements for each analyte. Calculate the MDL of each analyte using the referenced procedures (1).

9.3.3 For each analyte and surrogate, the mean accuracy, expressed as a percentage of the true value, should be 70-130% and the RSD should be < 30%. The MDLs should be sufficient to detect analytes at the regulatory levels. If these criteria are not met for an analyte, take remedial action and repeat the measurements for that analyte to demonstrate acceptable performance before samples are analyzed.

9.3.4 Develop and maintain a system of control charts to plot the precision and accuracy of analyte and surrogate measurements as a function of time. Charting of surrogate recoveries is an especially valuable activity since these are present in every sample and the analytical results will form a significant record of data quality.

9.4 LABORATORY REAGENT BLANKS (LRBs) -- With each batch of samples processed as a group within a work shift, analyze a laboratory reagent blank to determine the background system contamination. Any time a new batch of LSE cartridges or disks is used, or new supplies of other reagents are used, repeat the demonstration of low background described in Sect. 9.2.

9.5 With each batch of samples processed as a group within a work shift, analyze a single LFB containing each analyte of concern at a concentration as determined in Sect. 9.3. Evaluate the accuracy of the measurements (Sect. 9.3.3), and estimate whether acceptable MDLs can be obtained. If acceptable accuracy and MDLs cannot be achieved, the problem must be located and corrected before further samples are analyzed. Add these results to the ongoing control charts to document data quality.

9.6 Determine that the sample matrix does not contain materials that adversely affect method performance. This is accomplished by analyzing replicates of laboratory fortified matrix samples and ascertaining that the precision, accuracy, and MDLs of analytes are in the same range as obtained with LFBs. If a variety of different sample matrices are analyzed regularly, for example, drinking water from groundwater and surface water sources, matrix independence should be established for each.

9.7 With each set of field samples a FRB should be analyzed. The results of these analyses will help define contamination resulting from field sampling and transportation activities.

9.8 At least quarterly, replicates of LFBs should be analyzed to determine the precision of the laboratory measurements. Add these results to the ongoing control charts to document data quality.

9.9 At least quarterly, analyze a QCS from an external source. If measured analyte concentrations are not of acceptable accuracy (Sect. 9.3.3), check the entire analytical procedure to locate and correct the problem source.

9.10 Numerous other specific QC measures are incorporated into other parts of this procedure, and serve to alert the analyst to potential problems.

## 10. CALIBRATION AND STANDARDIZATION

10.1 Demonstration and documentation of acceptable initial calibration and system optimization are required before any samples are analyzed and is required intermittently during sample analysis as indicated by results of continuing calibration checks. After initial calibration is successful, a continuing calibration check is required at the beginning of each 8-hr period during which analyses are performed. Additional periodic calibration checks are good laboratory practice.

### 10.2 INITIAL CALIBRATION

10.2.1 Optimize the interface according to the manufacturer's instructions. This usually is accomplished on initial installation by flow injection with caffeine or benzidine and should utilize a mobile phase of 50% v/v acetonitrile: water. Major maintenance may require reoptimization.

10.2.2 Calibrate the MS mass and abundance scales using the calibration compound and manual (not automated) ion source tuning procedures specified by the manufacturer. Calibration must be accomplished while a 50% v/v acetonitrile: water mixture is pumped through the LC column and the optimized particle beam interface. For optimum long-term stability and precision, interface and ion source parameters should be set near the center of a broad signal plateau rather than at the peak of a sharp maximum (sharp maxima vary short term with particle beam interfaces and gradient elution conditions).

10.2.3 Fine tune the interface by making a series of injections into the LC column of a medium level CAL standard and adjusting the operating parameters until optimum sensitivity and precision are obtained for the maximum number of target compounds (6). Suggested additional operating conditions are:

mobile phase purge: helium at 30 mL/min continuous,

mobile phase flow rate: 0.3 mL/min through the column,

gradient elution: Hold for 1 min at 25% acetonitrile, then linearly program to ≈ 70% acetonitrile in 29 min, start data acquisition immediately,

post-column addition: acetonitrile at 0.1-0.7 mL/min, depending on the interface requirements. Maintain a minimum of 30% acetonitrile in the interface to improve system precision and possibly sensitivity,

desolvation chamber temperature: 45°-80°C,

ion source temperature: 250° - 290°C,

electron energy: 70 eV, and

scan range: 62-465 amu at 1-2 sec/scan.

10.2.4 The medium level standard (CAL) used in Sect. 10.2.3 should contain DFTPPO, or separately inject into the LC a 5-µL aliquot of the 100 ng/µL DFTPPO solution and acquire a mass spectrum that includes data for m/z 62-465. Use LC conditions that produce a narrow (at least ten scans per peak) symmetrical peak. If the spectrum does not meet all criteria (Table 1), the MS ion source must be retuned and adjusted to meet all criteria before proceeding with calibration. An average spectrum across the LC peak may be used to evaluate the performance of the system. Figure 2 represents the average composite spectrum obtained for DFTPPO from a multilaboratory study involving 5 different particle beam interfaces from 13 laboratories.

10.2.5 Inject a 5-μL aliquot of a medium concentration calibration solution, for example 50 ng/μL, and acquire and store data from m/z 62-465 with a total cycle time (including scan overhead time) of 1.5 secs or less. Cycle time should be adjusted to measure at least ten spectra during the elution of each LC peak.

10.2.6 Performance criteria for the medium calibration. Examine the stored LC/MS data with the data system software. Figure 3 shows an acceptable total ion chromatogram.

10.2.6.1 LC performance. 3,3'-dimethyl- and 3,3'- dimethoxybenzidine should be separated by a valley whose height is less than 25% of the average peak height of these two compounds. If the valley between them exceeds 25%, modify the gradient. If this fails, the LC column requires maintenance. (See Sect. 10.3.6)

10.2.6.2 Peak tailing -- Examine a total ion chromatogram and determine the degree of peak tailing. Severe tailing indicates a major problem and system maintenance is required to correct the problem. (See Sect. 10.3.6)

10.2.6.3 MS sensitivity -- Signal/noise in any analyte mass spectrum should be at least 3:1.

10.2.6.4 Column bleed -- Figure 1 shows an unacceptable chromatogram with column bleed. Figure 3 is the mass spectrum of dimethyloctadecylsilanol, a common stationary phase bleed product. If unacceptable column bleed is present, the column must be changed or conditioned to produce an acceptable background (Figure 4).

10.2.6.5 Coeluting compounds -- Compounds which coelute cannot be measured accurately because of carrier effects in the particle beam interface. Peaks must be examined carefully for coeluting substances and if coeluting compounds are present at greater than 10% the concentration of the target compound, conditions must be adjusted to resolve the components, the target compound must be flagged as positively biased, or isotope dilution calibration should be used.

10.2.7 If all performance criteria are met, inject a 5-μL aliquot of each of the other CAL solutions using the same LC/MS conditions.

10.2.8 The general method of calibration (external) is a second order regression of integrated ion abundances of the quantitation ions (Table 2) as a function of amount injected. For second order regression, a sufficient number of calibration points must be obtained to accurately determine the equation of the curve. For some individual analytes over a short concentration range, reasonable linearity may be observed and response factors may be used. Calculate response factors using the equation below. Second order regressions and response factor calculations are supported in acceptable LC/MS data system software (Sect. 6.16.5), and many other software programs.

$$RF = \frac{(A_x)}{(Q_x)}$$

where: $A_x$ = integrated abundance of the quantitation ion of the analyte.

$Q_x$ = quantity of analyte injected in ng or concentration units.

10.2.9 If response factors are used (i.e., linear calibration with the line going through the origin), calculate the mean RF from the analyses of the six CAL solutions for each analyte and surrogate. Calculate the standard deviation (SD) and the relative standard deviation (RSD) from each mean (M): RSD = 100 (SD/M). If the RSD of any analyte or surrogate mean RF exceeds 20%, either analyze additional aliquots of appropriate CAL solutions to obtain an acceptable RSD of RFs over the entire concentration range, take action to improve LC/MS performance, or use the second order regression calibration. (See Sect. 10.2.8)

10.3 CONTINUING CALIBRATION CHECK -- Verify the MS tune and initial calibration at the beginning of each 8-hr work shift during which analyses are performed using the following procedure:

10.3.1 Inject a 5-μL aliquot of the 100 ng/μL DFTPPO solution (this may be contained in the medium level CAL solution used in Sect. 10.3.2) and acquire a mass spectrum that includes data for m/z 62-465. If the spectrum does not meet all criteria (Table 1), the MS must be retuned and adjusted to meet all criteria before proceeding with the continuing calibration check.

10.3.2 Inject a 5-$\mu$L aliquot of a medium level CAL solution and analyze with the same conditions used during the initial calibration. One or more additional CAL solutions should be analyzed.

10.3.3 Demonstrate acceptable performance for the criteria shown in Sect. 10.2.6.

10.3.4 Determine that the absolute areas of the quantitation ions of the external standards and surrogate(s) have not changed by more than 20% from the areas measured during initial calibration. If these areas have changed by more than 20%, recalibration and other adjustments are necessary. These adjustments may require cleaning of the MS ion source, or other maintenance as indicated in Sect. 10.3.6, and recalibration. Control charts are useful aids in documenting system sensitivity changes.

10.3.5 Using the previously generated second order regression curve, calculate the concentrations in the medium level CAL solution and compare the results to the known values in the CAL solution. If calculated concentrations deviate by more than 20% from known values, recalibration of the system with the 6 CAL solutions is required. If response factors were used, calculate the RF for each analyte and surrogate from the data measured in the continuing calibration check. The RF for each analyte and surrogate must be within 20% of the mean value measured in the initial calibration.

10.3.6 Some possible remedial actions -- Major maintenance such as cleaning an ion source, cleaning quadrupole rods, etc. require returning to the initial calibration step.

10.3.6.1 Check and adjust LC and/or MS operating conditions; check the MS resolution, and calibrate the mass scale.

10.3.6.2 Replace the mobile phases with fresh solvents. Verify that the combined flow rate from the LC and post-column addition pumps is constant.

10.3.6.3 Flush the LC column with acetonitrile.

10.3.6.4 Replace LC column; this action will cause a change in retention times.

10.3.6.5 Prepare fresh CAL solutions and repeat the initial calibration step.

10.3.6.6 Clean the MS ion source, entrance lens, and rods (if a quadrupole).

10.3.6.7 Replace any components that leak.

10.3.6.8 Replace the MS electron multiplier or any other faulty components.

10.3.6.9 Clean the interface to eliminate plugged components and/or replace skimmers according to the manufacturer's instructions.

10.3.6.10 If automated peak areas are being used, verify values by manual integration.

10.3.6.11 Increasing ion source temperature can reduce peak tailing, but excessive ion source temperature can affect the quality of the spectra for some compounds.

10.3.6.12 Air leaks into the interface may affect the quality of the spectra (e.g. DFTPPO), especially when ion source temperatures are operated in excess of 280°.

10.4 CALIBRATION WITH ISOTOPE DILUTION (OPTIONAL) -- For samples with interfering matrix or coeluting peaks, the most reliable method for quantitation is the use of coeluting isotope labelled internal standards (7). Isotope dilution calibration will be limited by the availability and cost of the labelled species and the requirement that each analyte must coelute with the labelled internal standard. Because the labelled internal standard must coelute with the analyte, the quantitation ion for the internal standard must be larger than that of the analyte and not present in the analyte's mass spectrum. In addition, it must be verified that the labelled internal standard is not contaminated by its unlabelled counterpart.

10.4.1 A calibration curve encompassing the concentration range is prepared for each compound to be determined. The relative response (analyte integrated ion abundances to labelled integrated ion abundance) vs. amount of analyte injected is plotted using linear regression. A minimum of five data points are employed for this type of calibration.

10.4.2 To calibrate, inject a 5.0 µL aliquot of each of the calibration standards (Sect. 7.12.1) and compute the relative response (analyte integrated ion abundances to labelled compound integrated ion abundance). Plot this versus the amount of analyte injected by linear regression. This plotted line or the equation of this line should be used for quantitative calculations. Unless this line goes through the origin, the response factors at each point will not be constant and therefore, average response factors cannot be used. These calculations are supported in acceptable LC/MS data system software (Sect. 6.15.5), and in many other software programs.

10.4.3 Follow Sect. 10.3 to verify calibration at the beginning of each 8-hr work shift by injecting a 5.0 µL aliquot of a medium CAL solution and analyze it with the same conditions used during the initial calibration. Using the previously generated first order regression line (relative response versus amount of analyte injected), calculate the concentrations in the medium level CAL solution and compare the results to the known values in the CAL solution. If calculated concentrations deviate by more than 20% from known values, recalibration of the system with the CAL solutions, containing the isotopically labelled analogues, is required.

## 11. PROCEDURE

11.1 The extraction procedure depends on the analytes selected and the nature of the sample. LSE (cartridge or disk) is limited to particulate-free water, e.g., drinking water. Consult Tables 3-5 to determine which analytes are amenable to liquid-solid and liquid-liquid extractions. Sect. 11.2 provides the LSE procedure using cartridges and Sect. 11.3 provides the LSE procedure using disks. Sect. 11.4 provides the procedure for LLE. After the extraction is complete, proceed to Sect. 11.5 to continue with the method.

### 11.2 LIQUID-SOLID EXTRACTION (LSE) PROCEDURE USING CARTRIDGES (This procedure may be manual or automated).

11.2.1 Set up the extraction apparatus shown in Figure 5. The reservoir is not required but recommended for convenient operation. Water drains from the reservoir through the LSE cartridge and into a syringe needle which is inserted through a rubber stopper into the suction flask. A slight vacuum of 13 cm (5 in) of mercury is used during all operations with the apparatus. The pressure used is critical as a vacuum greater than 13 cm may result in poor precision. About 2 hr is required to draw a liter of water through the cartridge, but faster flow rates are acceptable if precision and accuracy are acceptable. The use of robotic extraction systems is acceptable if equivalent MDLs, precision and accuracy are obtained.

11.2.2 Mark the water meniscus on the side of the sample bottle for later determination of the sample volume. A 1-L sample is recommended. Pour the water sample into the 2-L separatory funnel with the stopcock closed. Adjust the pH to 7.0 by the dropwise addition of hydrochloric acid or 1 N sodium hydroxide. Residual chlorine must not be present, as a reducing agent should have been added at the time of sampling. For extractions using $C_{18}$ cartridges, add 0.01 M ammonium acetate (0.77 g in 1 L) to the water sample and mix until homogeneous. Add a 10-µL aliquot of the fortification solution for surrogates, and mix until homogeneous. The concentration of surrogates in the water should be 10-50 times the instrument detection limit.

11.2.3 Flush each cartridge with two 10-mL aliquots of methanol, letting the cartridge drain dry after each flush. This solvent flush may be accomplished by adding methanol directly to the solvent reservoir in Figure 5. Add 10 mL of reagent water to the solvent reservoir, but before the reagent water level drops below the top edge of the packing in the LSE cartridge, open the stopcock of the separatory funnel and begin adding sample water to the solvent reservoir. Close the stopcock when an adequate amount of sample is in the reservoir.

11.2.4 Periodically open the stopcock and drain a portion of the sample water into the solvent reservoir. The water sample will drain into the cartridge, and from the exit into the suction flask. Maintain the packing material in the cartridge immersed in water at all times. Wash the separatory funnel and cartridge with 10 mL of reagent water, and draw air through the cartridge for 10 min.

11.2.5 Transfer the LSE cartridge to the elution apparatus shown in Figure 5B. Wash the 2-L separatory funnel with 15 mL of methanol, close the stopcock of the 100-mL separatory funnel of the elution apparatus, and elute the cartridge with two 7.5-mL aliquots of the methanol washings. Concentrate the extract to the desired volume under a gentle stream of nitrogen. Record the exact volume of the extract.

11.2.5.1 If isotope dilution calibration is used, spike the extract with the isotopically labelled standards prior to solvent evaporation. The concentration of these isotopically labelled compounds after the desired extract volume is reached should be the same as the concentration in each CAL solution.

11.2.6 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

## 11.3 LIQUID-SOLID EXTRACTION (LSE) PROCEDURE USING DISKS (This procedure may be manual or automated).

11.3.1 Mark the water meniscus on the side of the sample bottle for later determination of the sample volume. A 1-L sample is recommended. Pour the water sample into the 2-L separatory funnel with the stopcock closed. Adjust the pH to 7.0 by the dropwise addition of hydrochloric acid or 1 N sodium hydroxide. Residual chlorine must not be present because a reducing agent should have been added at the time of sampling. For extractions using $C_{18}$ disks, add 0.01 M ammonium acetate (0.77 g in 1 L) to the water sample and mix until homogeneous. Add a 10-$\mu$L aliquot of the fortification solution for surrogates, and mix until homogeneous. The concentration of surrogates in the water should be 10-50 times the instrument detection limit.

### 11.3.2 Preparation of Disks

11.3.2.1 Insert the disk into the 47 mm filter apparatus (See Figure 6). Wash and pre-wet the disk with 10 mL methanol (MeOH) by adding the MeOH to the disk and allowing it to soak for about a min, then pulling most of the remaining MeOH through. A layer of MeOH must be left on the surface of the disk, which should not be allowed to go dry from this point until the end of the sample extraction. THIS IS A CRITICAL STEP FOR A UNIFORM FLOW AND GOOD RECOVERY.

11.3.2.2 Rinse the disk with 10 mL reagent water by adding the water to the disk and pulling most through, again leaving a layer of water on the surface of the disk.

11.3.3 Add the water sample to the reservoir and turn on the vacuum to begin the extraction. Full aspirator vacuum may be used. Particulate-free water may pass through the disk in as little as 10 min or less. Extract the entire sample, draining as much water from the sample container as possible.

11.3.4 Remove the filtration top from the flask, but do not disassemble the reservoir and fritted base. Empty the water from the flask, and insert a suitable sample tube to contain the eluant. The only constraint on the sample tube is that it must fit around the drip tip of the fritted base. Reassemble the apparatus.

11.3.5 Add 5 mL MeOH to the sample bottle, and rinse the inside walls thoroughly. Allow the MeOH to settle to the bottom of the bottle, and transfer to the disk with a disposable pipet, rinsing the sides of the glass filtration reservoir in the process. Pull about half of the MeOH through the disk, release the vacuum, and allow the disk to soak for a minute. Pull the remaining MeOH through the disk.

11.3.6 Repeat the above step twice. Concentrate the combined extracts to the desired volume under a gentle stream of nitrogen. Record the exact volume of the extract. (Preliminary investigation indicates that acetonitrile is a better extraction solvent for rotenone when extracting water, containing high levels of particulate matter, with LSE disks.)

11.3.6.1 If isotope dilution calibration is used, spike the extract with the isotopically labelled standards prior to solvent evaporation. The concentration of these isotopically labelled compounds after the desired extract volume is reached should be the same as the concentration in each CAL solution.

11.3.7 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

## 11.4 LIQUID-LIQUID EXTRACTION (LLE) PROCEDURE

11.4.1 Mark the water meniscus on the side of the sample bottle for later determination of the sample volume. A 1-L sample is recommended. Pour the water sample into a 2-L separatory funnel with the stopcock closed. Residual chlorine should not be present as a reducing agent should have been added at the time of sampling. Add a 10-μL aliquot of the fortification solution for surrogates, and mix until homogeneous. The concentration of surrogates in the water should be 10-50 times the instrument detection limit.

11.4.2 Adjust the pH of the sample to 7.0 by dropwise addition of hydrochloric acid or 1 N sodium hydroxide. Add 100 g of sodium chloride to the sample and shake to dissolve the salt.

11.4.3 Add 60 mL of methylene chloride to the sample bottle, shake, and transfer the solvent to the separatory funnel and extract the sample by vigorously shaking the funnel for 2 min with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one-third the volume of the solvent layer, mechanical techniques must be employed to complete the phase separation. The optimum technique depends on the sample, but may include stirring, filtration of the emulsion through glass wool, centrifuging, etc. Collect the methylene chloride extract in a 500-mL Erlenmeyer flask.

11.4.4 Add a second 60-mL volume of methylene chloride and repeat the extraction a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

11.4.5 Assemble a K-D concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Dry the extract by pouring it through a solvent-rinsed drying column containing about 10 cm of anhydrous sodium sulfate. Collect the extract in the K-D concentrator, and rinse the column with 20-30 mL of methylene chloride.

11.4.6 Add 1 or 2 clean boiling stones to the evaporative flask and attach a macro Snyder column. Pre-wet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath, 65-70°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15-20 min. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of the liquid reaches 2 mL, add 20 mL of methanol through the Snyder column using a syringe and needle. Raise the temperature of the hot water bath to 90°C, and concentrate the sample to about 2 mL. Concentrate the extract to the desired volume under a gentle stream of nitrogen. Record the exact volume of the concentrated extract.

11.4.6.1 If isotope dilution calibration is used, spike the extract with the isotopically labelled standards prior to solvent evaporation. The concentration of these isotopically labelled compounds after the desired extract volume is reached should be the same as the concentration in each CAL solution.

11.4.7 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

## 11.5 LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY (LC/MS)

11.5.1 Analyze a 5-$\mu$L aliquot with the LC/MS system under the same conditions used for the initial and continuing calibrations (Sect. 10.2).

## 11.6 IDENTIFICATION OF ANALYTES

11.6.1 At the conclusion of data acquisition, use the system software to display the chromatogram, mass spectra and retention times of the peaks in the chromatogram.

11.6.2 Identify a sample component by comparison of its mass spectrum (after background subtraction) to a reference spectrum in the user-created data base. The LC retention time of the sample component should be within 10 sec of the time observed for that same compound when a calibration solution was analyzed. In general, all ions that are present above 10% relative abundance in the mass spectrum of the standard should be present in the mass spectrum of the sample component and should agree within absolute 20%. For example, if an ion has a relative abundance of 30% in the standard spectrum, its abundance in the sample spectrum should be in the range of 10 to 50%. Some ions, particularly the molecular ion, are of special importance, and should be evaluated even if they are below 10% relative abundance.

11.6.3 Use the data system software to examine the ion abundances of components of the chromatogram. If any ion abundance exceeds the system working range, dilute the sample aliquot and analyze the diluted aliquot.

11.6.4 Identification is hampered when sample components are not resolved chromatographically and produce mass spectra containing ions contributed by more than one analyte. When LC peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or vallies between two or more maxima), appropriate analyte spectra and background spectra can be selected by examining plots of characteristic ions for tentatively identified components. When analytes coelute (i.e., only one LC peak is apparent), the identification criteria can be met but each analyte spectrum will contain extraneous ions contributed by the coeluting compound.

11.6.5 Structural isomers that produce very similar mass spectra can be explicitly identified only if they have sufficiently different LC retention times. (See Sect. 10.2.6.1) Acceptable resolution is achieved if the height of the valley between two isomer peaks is less than 25% of the average height of the two peak heights. Otherwise, structural isomers are identified as isomeric pairs.

11.6.6 Background components appear in variable quantities in laboratory and field reagent blanks, and generally subtraction of the concentration in the blank from the concentration in the sample is not recommended because the concentration of the background in the blank is highly variable. If method analytes appear in the blank, then resample.

## 12. DATA ANALYSIS AND CALCULATIONS

12.1 Complete chromatographic resolution is necessary for accurate and precise measurements of analyte concentrations. Compounds which coelute cannot be measured accurately because of carrier effects in the particle beam interface (2). Peaks must be examined carefully for coeluting substances and if coeluting compounds are present at greater than 10% the concentration of the target compound, either conditions must be adjusted to resolve the components, or the target compound must be removed from the list of quantitative analytes.

12.2 Use the LC/MS system software or other available proven software to compute the concentrations of the analytes and surrogates from the second order regression curves. Manual verification of automated integration is recommended.

12.2.1 For isotope dilution calculations, use the first order plot of relative response (analyte integrated ion abundances to labelled integrated ion abundance) vs. amount of analyte injected or the equation of the line to compute concentrations. If the plotted line does not go through the origin, response factors will not be constant at each calibration point; therefore, average response factors cannot be used.

12.3 If appropriate, calculate analyte and surrogate concentrations from response factors and the following equation.

$$C_x = \frac{(A_x)\ V_e}{RF\ V\ V_i}$$

where:

$C_x$ = Concentration of analyte or surrogate in $\mu$g/L in the water sample.

$A_x$ = integrated abundance of the quantitation ion of the analyte in the sample.

$V$ = original water sample volume in liters.

$RF$ = mean response factor of analyte from the initial calibration.

$V_e$ = volume of final extract in $\mu$L

$V_i$ = injection volume in $\mu$L

## 13. METHOD PERFORMANCE

13.1 Single laboratory accuracy and precision data (Tables 3-5) for each listed analyte were obtained. Five to seven 1-L aliquots of reagent water containing approximately 5 times the MDL of each analyte were analyzed with this procedure. (For these experiments, the final extract volume was 0.5 mL.)

13.1.2 With these data, MDLs were calculated using the formula:

$$MDL = S\, t_{(n-1, 1-alpha = 0.99)}$$

where:

$t_{(n-1, 1-alpha = 0.99)}$ =

Student's t value for the 99% confidence level with n-1 degrees of freedom, where n = number of replicates, and S = standard deviation of replicate analyses.

13.2 A multilaboratory (12 laboratories) validation of the determinative step was done for four of the analytes (benzidine - BZ, 3,3'-dimethoxybenzidine - MB, 3,3'-dimethylbenzidine - LB, 3,3'-dichlorobenzidine - DB). Table 6 gives the results from this study for single laboratory precision, overall laboratory precision, and overall laboratory accuracy. The two concentration levels shown represent the two extremes of the concentration range studied.

## 14. POLLUTION PREVENTION

14.1 Although this method allows the use of either LLE or LSE, LSE is highly recommended whenever possible. Only small amounts of methanol are used with this procedure as compared to much larger amounts of methylene chloride used for LLE. All other compounds used are neat materials used to prepare standards and sample preservatives. All compounds are used in small amounts and pose minimal threat to the environment if properly disposed.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less Is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C., 20036.

## 15. WASTE MANAGEMENT

15.1 There are generally no waste management problems involved with discarding spent or left over samples in this method since most often the sample matrix is drinking water. If a sample is analyzed which appears to be highly contaminated with chemicals, analyses should be carried out to assess the type and degree of contamination so that the samples may be discarded properly. All other expired standards should be discarded properly. It is the laboratory's responsibility to comply with all applicable regulations for waste disposal. The Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" also available from the American Chemical Society at the address in Sect. 14.2.

## 16. REFERENCES

1. Glaser, J.A., D.L. Foerst, G.D. McKee, S.A. Quave, and W.L. Budde, "Trace Analyses for Wastewaters," Environ. Sci. Technol. 1981 **15**, 1426-1435.

2. Bellar, T.A., T.D. Behymer, and W.L. Budde, "Investigation of Enhanced Ion Abundances from a Carrier Process in High-Performance Liquid Chromatography Particle Beam Mass Spectrometry," J. Am. Soc. Mass Spectrom., 1990, 1, 92-98.

3. "Carcinogens - Working With Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.

4. "OSHA Safety and Health Standards, General Industry," (29CFR1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).

5. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

6. Behymer, T.D., T.A. Bellar, and W.L. Budde, "Liquid Chromatography/Particle Beam/Mass Spectrometry of Polar Compounds of Environmental Interest," Anal. Chem., 1990, 62, 1686-1690.

7. Ho, J.S., T.D. Behymer, W.L. Budde, and T.A. Bellar, "Mass Transport and Calibration in Liquid Chromatography/Particle Beam/Mass Spectrometry," J. Am. Soc. Mass Spectrom., 1992, 3, 662-671.

## 17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

### TABLE 1. ION ABUNDANCE CRITERIA FOR BIS(PERFLUOROPHENYL)PHENYLPHOSPHINE OXIDE (DECAFLUOROTRIPHENYLPHOSPHINE OXIDE, DFTPPO)

| Mass (M/z) | Relative Abundance Criteria | Purpose of Checkpoint[1] |
|---|---|---|
| 77 | present, major ion | low mass sensitivity |
| 168 | present, major ion | mid-mass sensitivity |
| 169 | 4-10% of 168 | mid-mass resolution and isotope ratio |
| 271 | present, major ion | base peak |
| 365 | 5-10% of base peak | baseline threshold check |
| 438 | present | important high mass fragment |
| 458 | present | molecular ion |
| 459 | 15-24% of mass 458 | high mass resolution and isotope ratio |

[1] All ions are used primarily to check the mass measuring accuracy of the mass spectrometer and data system, and this is the most important part of the performance test. The resolution checks, which include natural abundance isotope ratios, constitute the next most important part of the performance test. The correct setting of the baseline threshold, as indicated by the presence of low intensity ions, is the next most important part of the performance test. Finally, the ion abundance ranges are designed to encourage some standardization of fragmentation patterns.

## TABLE 2. RETENTION TIME DATA AND QUANTITATION IONS FOR METHOD ANALYTES

| Compound | Retention Time(min:sec) $\underline{A}^a$ | $\underline{B}^b$ | Quantitation Ion (m/z) |
|---|---|---|---|
| benzidine | 4.3 | 4.9 | 184 |
| benzoylprop ethyl | 24.8 | 31.3 | 105 |
| caffeine | 1.4 | 1.6 | 194 |
| carbaryl | 10.1 | 14.7 | 144 |
| o-chlorophenyl thiourea | 2.7 | 3.0 | 151 |
| 3,3'-dichlorobenzidine | 16.6 | 22.7 | 252 |
| 3,3'-dimethoxybenzidine | 8.1 | 11.5 | 244 |
| 3,3'-dimethylbenzidine | 8.5 | 12.4 | 212 |
| diuron | 11.0 | 16.1 | 72 |
| ethylene thiourea | 1.2 | 1.4 | 102 |
| linuron | 16.0 | 21.9 | 161 |
| rotenone | 21.1 | 27.4 | 192 |
| siduron | 14.8 | 20.6 | 93 |
| Surrogates:[c] | | | |
| benzidine-$D_8$ | 4.2 | 4.8 | 192 |
| caffeine-$^{15}N_2$ | 1.3 | 1.6 | 196 |
| 3,3'-dichlorobenzidine-$D_6$ | 16.5 | 22.6 | 258 |
| bis(perfluorophenyl)-phenylphosphine oxide | 22.0 | 28.9 | 271 |

---

[a] These retention times were obtained on a Hewlett-Packard 1090 liquid chromatograph with a Waters C18 Novapak 15 cm x 2 mm column using gradient conditions given in Sect. 10.2.3.

[b] These retention times were obtained on a Waters 600 MS liquid chromatograph with a Waters C18 Novapak 15 cm x 2 mm column using gradient conditions given in Sect. 10.2.3.

[c] These compounds cannot be used if unlabelled compounds are present (See Sect. 4.1).

## TABLE 3. ACCURACY AND PRECISION DATA FROM SIX DETERMINATIONS OF THE METHOD ANALYTES IN REAGENT WATER USING LIQUID-LIQUID EXTRACTION

| Compound | True Conc. (μg/L) | Mean Observed Conc. (μg/L) | Std. Dev. (μg/L) | Rel. Std. Dev. (%) | Mean Method Accuracy (% of True Conc.) | Method Detection Limit (MDL) (μg/L) |
|---|---|---|---|---|---|---|
| benzidine | 22.9 | 20.5 | 0.8 | 3.3 | 89.6 | 2.5 |
| benzoylprop ethyl | 32.5 | 33.0 | 1.1 | 3.3 | 101.6 | 3.7 |
| caffeine | 14.4 | 10.5 | 0.9 | 6.3 | 72.6 | 3.1 |
| carbaryl | 56.6 | 52.2 | 2.9 | 5.1 | 92.3 | 9.8 |
| o-chlorophenyl thiourea | 32.6 | 15.3 | 2.2 | 6.8 | 47.0 | 7.4* |
| 3,3'-dichlorobenzidine | 24.8 | 21.7 | 0.7 | 2.9 | 89.6 | 2.4 |
| 3,3'-dimethoxybenzidine | 31.6 | 29.2 | 2.3 | 7.3 | 92.3 | 7.7 |
| 3,3'-dimethylbenzidine | 31.7 | 31.8 | 1.0 | 3.1 | 100.4 | 3.3 |
| diuron | 25.0 | 26.2 | 1.3 | 5.1 | 104.8 | 4.4 |
| ethylene thiourea | 32.0 | 0.0 | 0.0 | 0.0 | 0.0 | * |
| linuron | 95.0 | 89.5 | 3.9 | 4.1 | 94.2 | 13.1 |
| monuron | 31.2 | 31.8 | 1.2 | 3.8 | 101.9 | 4.0 |
| rotenone | 50.3 | 44.9 | 9.4 | 18.8 | 89.3 | 31.6 |
| siduron | 27.9 | 29.6 | 1.4 | 5.2 | 106.3 | 4.7 |

* - Recovery was not in the 70-130% range (See Sect. 9.3.3)

TABLE 4. ACCURACY AND PRECISION DATA FROM SEVEN DETERMINATIONS OF THE METHOD ANALYTES IN REAGENT WATER USING LIQUID-SOLID EXTRACTION ($C_{18}$ LSE CARTRIDGE)

| Compound | True Conc. ($\mu$g/L) | Mean Observed Conc. ($\mu$g/L) | Std. Dev. ($\mu$g/L) | Rel. Std. Dev. (%) | Mean Method Accuracy (% of True Conc.) | Method Detection Limit (MDL) ($\mu$g/L) |
|---|---|---|---|---|---|---|
| benzidine | 22.9 | 12.2 | 1.7 | 13.7 | 53.2 | 5.3* |
| benzoylprop ethyl | 32.5 | 29.3 | 2.0 | 6.9 | 90.2 | 6.3 |
| caffeine | 14.4 | 6.4 | 1.4 | 21.4 | 44.2 | 4.4* |
| carbaryl | 56.6 | 53.9 | 1.8 | 3.3 | 95.2 | 5.7 |
| o-chlorophenyl thiourea | 32.6 | 0.0 | 0.0 | 0.0 | 0.0 | * |
| 3,3'-dichlorobenzidine | 5.0 | 4.4 | 0.4 | 10.0 | 89.6 | 1.4 |
| 3,3'-dimethoxybenzidine | 31.6 | 25.5 | 1.8 | 7.1 | 80.8 | 5.7 |
| 3,3'-dimethylbenzidine | 31.7 | 31.4 | 1.0 | 3.1 | 99.0 | 3.0 |
| diuron | 25.0 | 24.4 | 1.4 | 5.6 | 97.6 | 4.4 |
| ethylene thiourea | 32.0 | 0.0 | 0.0 | 0.0 | 0.0 | * |
| linuron | 95.0 | 88.9 | 4.8 | 5.4 | 93.6 | 15.1 |
| monuron | 31.2 | 30.5 | 2.9 | 9.6 | 97.8 | 9.1 |
| rotenone | 50.3 | 45.0 | 2.4 | 5.4 | 89.6 | 7.5 |
| siduron | 27.9 | 24.8 | 2.0 | 7.9 | 88.9 | 6.3 |

* - Recovery was not in the 70-130% range (See Sect. 9.3.3)

TABLE 5. ACCURACY AND PRECISION DATA FROM SIX DETERMINATIONS OF THE METHOD ANALYTES IN REAGENT WATER USING LIQUID-SOLID EXTRACTION (NEUTRAL POLYSTYRENE/DIVINYLBENZENE POLYMER LSE DISK)

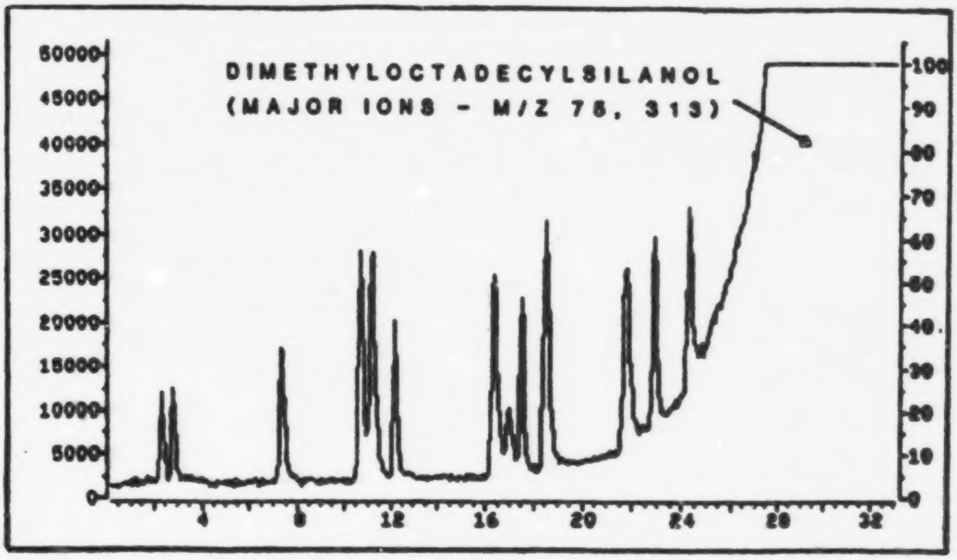
| Compound | True Conc. (µg/L) | Mean Observed Conc. (µg/L) | Std. Dev. (µg/L) | Rel. Std. Dev. (%) | Mean Method Accuracy (% of True Conc.) | Method Detection Limit (MDL) (µg/L) |
|---|---|---|---|---|---|---|
| benzidine | 22.9 | 24.7 | 2.4 | 9.8 | 108.0 | 8.1 |
| benzoylprop ethyl | 32.5 | 31.1 | 3.0 | 9.6 | 95.8 | 10.1 |
| caffeine | 14.4 | 0.7 | 0.5 | 72.5 | 5.2 | 1.8* |
| carbaryl | 56.6 | 59.5 | 4.7 | 7.9 | 105.1 | 15.8 |
| o-chlorophenyl thiourea | 32.6 | 0.0 | 0.0 | 0.0 | 0.0 | * |
| 3,3'-dichlorobenzidine | 5.0 | 5.0 | 0.5 | 9.4 | 101.7 | 1.6 |
| 3,3'-dimethoxybenzidine | 31.6 | 32.8 | 2.2 | 6.7 | 103.8 | 7.4 |
| 3,3'-dimethylbenzidine | 31.7 | 31.5 | 2.1 | 6.7 | 99.4 | 7.1 |
| diuron | 25.0 | 26.1 | 1.8 | 7.0 | 104.5 | 6.1 |
| ethylene thiourea | 32.0 | 0.0 | 0.0 | 0.0 | 0.0 | * |
| linuron | 95.0 | 97.9 | 8.7 | 9.0 | 103.0 | 29.3 |
| monuron | 31.2 | 34.4 | 2.5 | 7.3 | 110.4 | 8.4 |
| rotenone | 50.3 | 40.5 | 6.0 | 14.8 | 80.5 | 20.2 |
| siduron | 27.9 | 26.8 | 1.0 | 3.6 | 96.1 | 3.4 |

* - Recovery was not in the 70-130% range (See Sect. 9.3.3)

TABLE 6. MEAN RECOVERIES, MULTILABORATORY PRECISION AND ESTIMATES OF SINGLE ANALYST PRECISION FOR THE MEASUREMENTS OF FOUR BENZIDINES BY LC/PB/MS

| | 10 μg/mL | | | 100 μg/mL | | |
|---|---|---|---|---|---|---|
| Compound | Recovery % | RSD Multi-Lab | RSD Single Analyst | Recovery % | RSD Multi-Lab | RSD Single Analyst |
| BZ | 96 | 10 | 5.6 | 97 | 10 | 9.1 |
| MB | 104 | 20 | 18 | 95 | 10 | 7.0 |
| LB | 98 | 14 | 10 | 97 | 8.6 | 4.9 |
| DB | 96 | 18 | 9.4 | 97 | 9.1 | 4.6 |

C18 COLUMN FOLLOWING EXPOSURE TO AMMONIUM ACETATE



C18 COLUMN MAINTAINED WITH ACETONITRILE FLUSHING

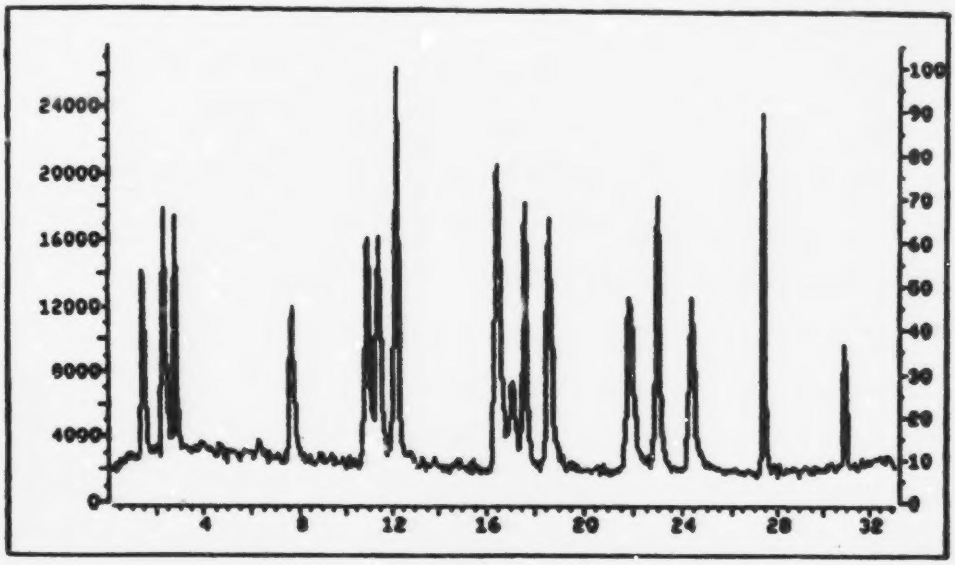


FIGURE 1. Unacceptable chromatogram with column bleed and acceptable chromatogram following column flushing.

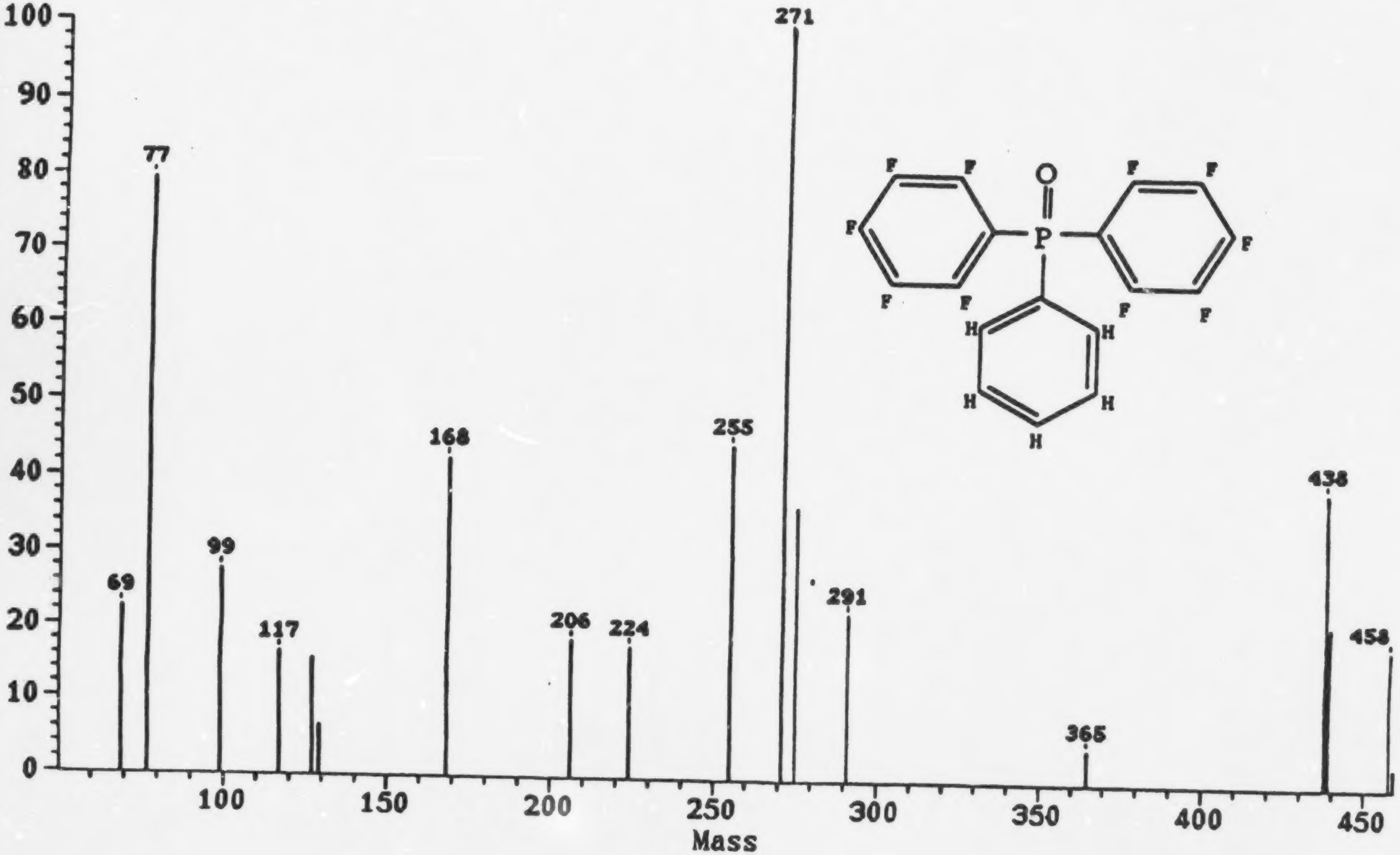


Figure 2. Average spectrum of DFTPPO from multilaboratory study.

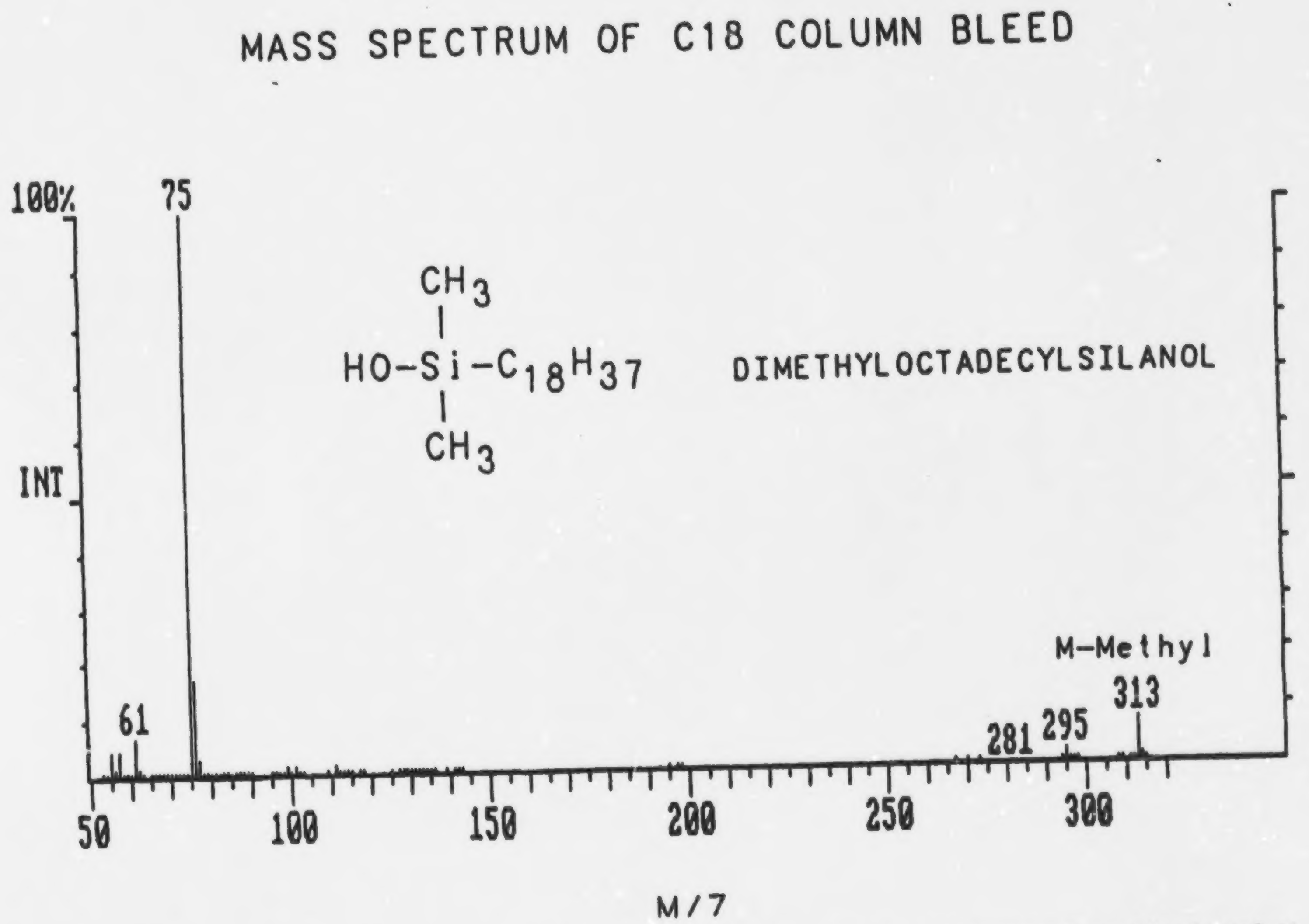


FIGURE 3. Mass spectrum of dimethyloctadecylsilanol, a common stationary phase bleed product.

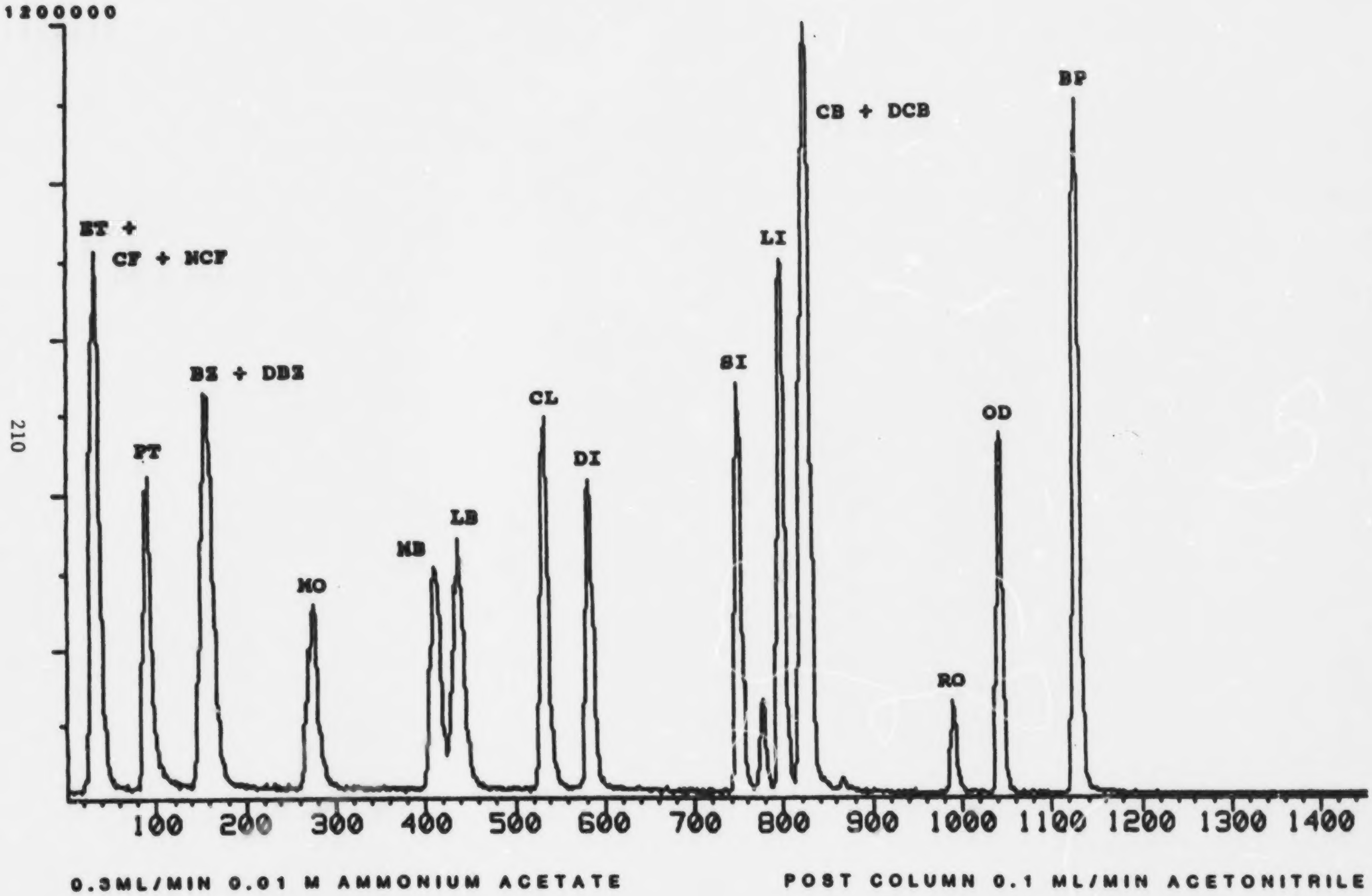


FIGURE 4. Total ion chromatogram of analytes and surrogates (140-950 ng injected).

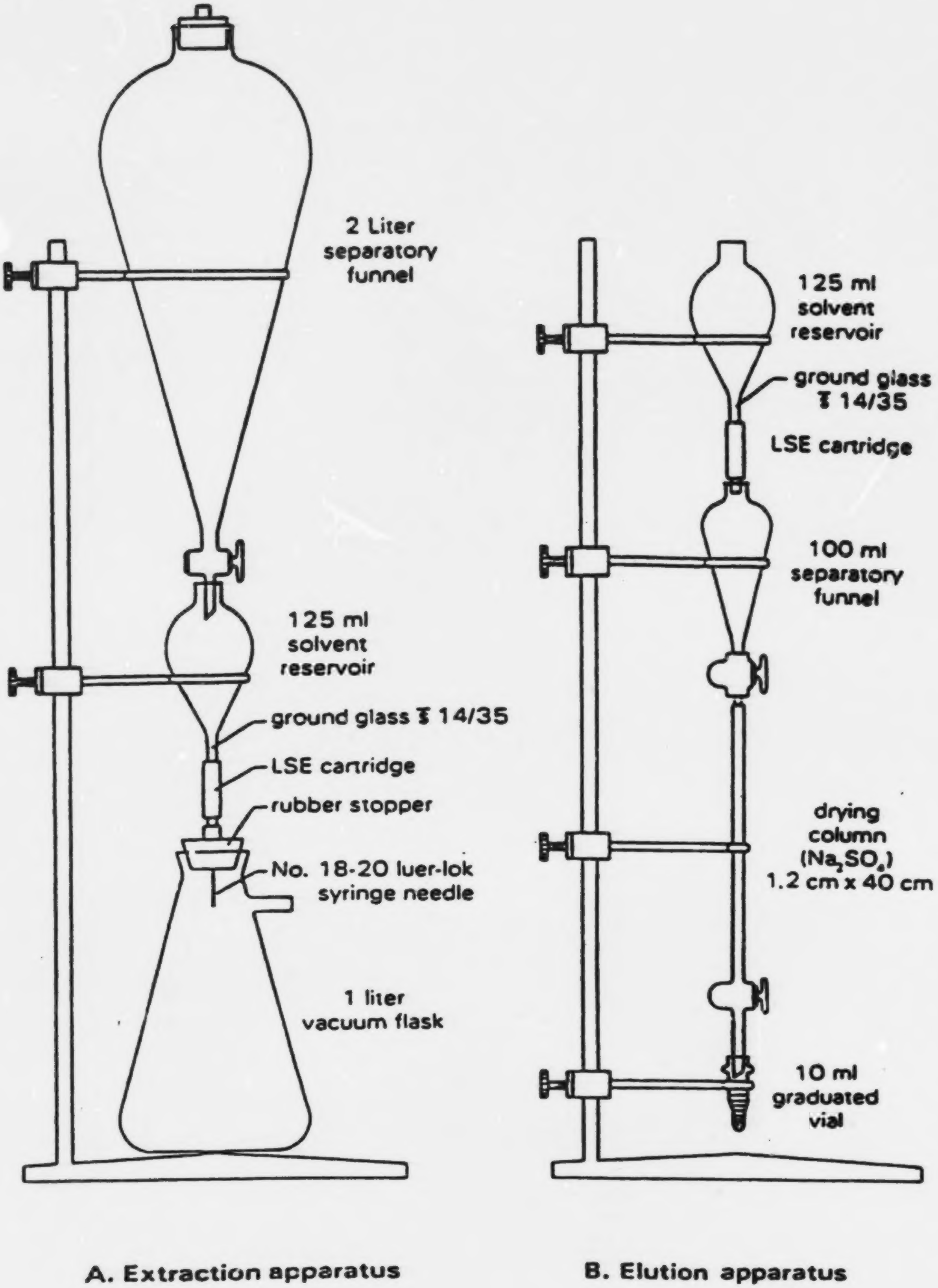

2 Liter separatory funnel
125 ml solvent reservoir
ground glass ₮ 14/35
LSE cartridge
rubber stopper
No. 18-20 luer-lok syringe needle
1 liter vacuum flask
A. Extraction apparatus
125 ml solvent reservoir
ground glass ₮ 14/35
LSE cartridge
100 ml separatory funnel
drying column ($Na_2SO_4$) 1.2 cm x 40 cm
10 ml graduated vial
B. Elution apparatus

FIGURE 5. Schematic diagram of a liquid-solid extaction (LSE) apparatus.

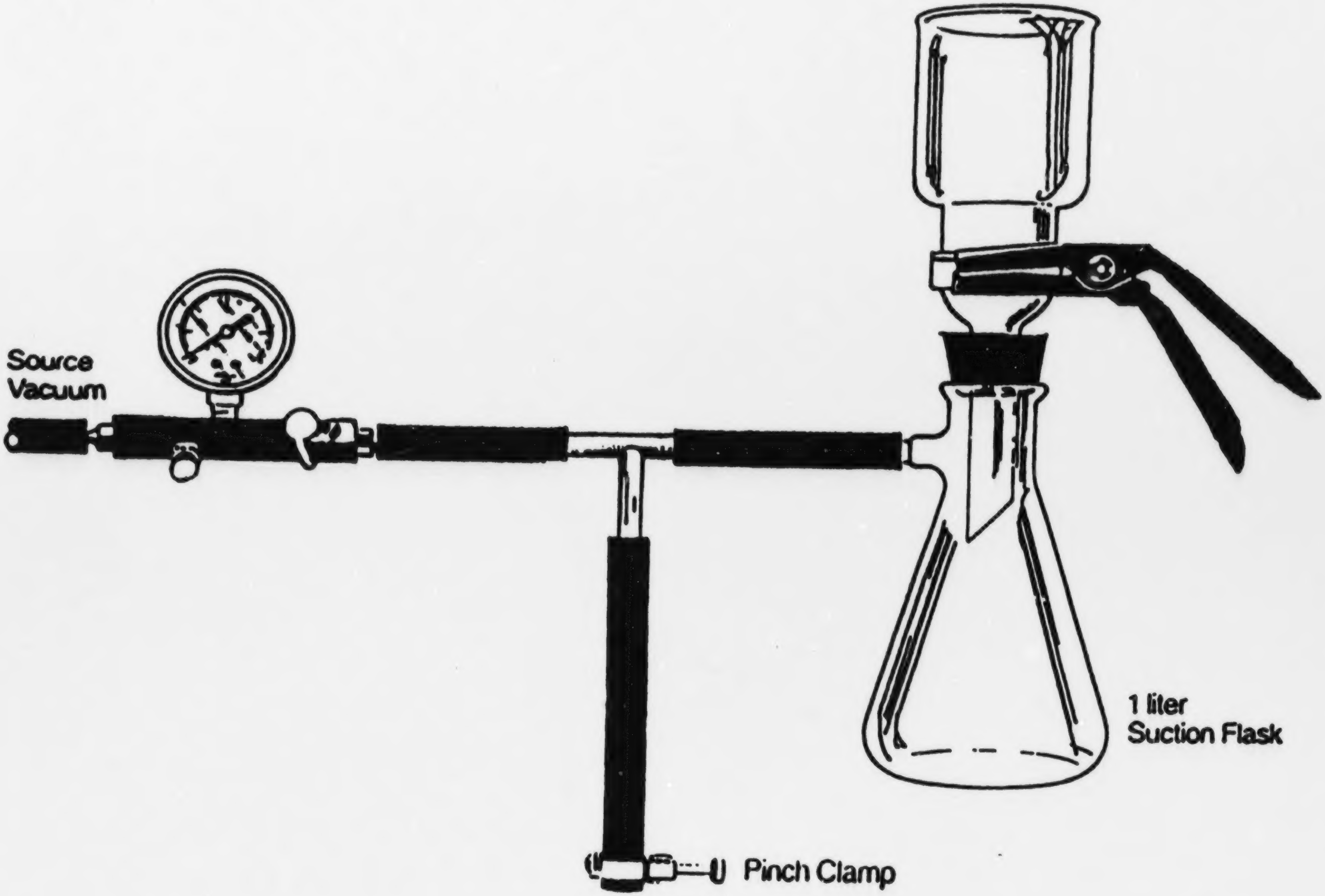

Source
Vacuum
1 liter
Suction Flask
Pinch Clamp

Figure 6. Schematic diagram of liquid-solid disk extraction apparatus.

# METHOD 554. DETERMINATION OF CARBONYL COMPOUNDS IN DRINKING WATER BY DINITROPHENYLHYDRAZINE DERIVATIZATION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Revision 1.0

August 1992

James W. Eichelberger

W. J. Bashe (Technology Applications, Incorporated)

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U. S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

## METHOD 554

## DETERMINATION OF CARBONYL COMPOUNDS IN DRINKING WATER BY DINITROPHENYLHYDRAZINE DERIVATIZATION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

### 1. SCOPE AND APPLICATION

1.1 This is a high performance liquid chromatographic (HPLC) method optimized for the determination of selected carbonyl compounds in finished drinking water and raw source water. The analytes applicable to this method are partitioned from the water onto a reverse phase $C_{18}$ bonded silica packed cartridge, then eluted with ethanol. Liquid-solid extraction disks may also be used for this purpose. Single-laboratory accuracy and precision data have been generated for the following compounds:

| Analyte | Chemical Abstract Services Registry Number |
|---|---|
| Formaldehyde | 50-00-0 |
| Acetaldehyde | 75-07-0 |
| Propanal | 123-38-6 |
| Butanal | 123-72-8 |
| Pentanal | 110-62-3 |
| Hexanal | 66-25-1 |
| Heptanal | 111-71-7 |
| Octanal | 124-13-0 |
| Nonanal | 124-19-6 |
| Decanal | 112-31-2 |
| Cyclohexanone | 108-94-1 |
| Crotonaldehyde | 123-73-9 |

1.2 The method detection limits (MDLs) for the analytes are listed in Tables 1 and 2. The MDL is defined as the statistically calculated minimum amount that can be measured with 99% confidence that the reported value is greater than zero (1). The MDLs for a specific sample may differ from that of the standard matrix and by the volume of sample used in the procedure. For the listed analytes, MDLs range from 3.0 to 69.0 $\mu$g/L.

1.3 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC and in the interpretation of chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method, using the procedure described in Sect. 11.

## 2. SUMMARY OF METHOD

2.1 A measured volume of aqueous sample, approximately 100 mL, is buffered to pH 3 and the analytes are derivatized at 40°C for 1 hr with 2,4-dinitrophenylhydrazine (DNPH). The derivatives are extracted from the water by passing the sample through a series of 3 cartridges each of which contains 500 mg of a chemically bonded $C_{18}$ organic phase (liquid-solid extraction, LSE). The solid sorbent cartridges are then eluted with 10 mL of ethanol. LSE disks may also be used as long as all the (QC) criteria specified in Sect. 9 of this method are met. Liquid chromatographic conditions are described which permit the separation and measurement of the carbonyl compounds in the extract by absorbance detection at 360 nm.

## 3. DEFINITIONS

3.1 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents and reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.2 FIELD REAGENT BLANK (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.3 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.4 LABORATORY FORTIFIED MATRIX SAMPLE (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.5 STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.6 PRIMARY DILUTION STANDARD SOLUTION (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.7 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution and stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.8 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

## 4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by analyzing laboratory reagent blanks as described in Sect. 9.2.

4.1.1 Glassware must be scrupulously cleaned as soon as possible after use by rinsing with the last solvent used. This should be followed by detergent washing with hot water, and rinses with tap water and distilled water. Glassware should then be drained, dried, and heated in a laboratory oven at 130°C for several hours before use. Solvent rinses with methanol may be substituted for the oven heating. After drying and cooling, glassware should be stored in a clean environment to prevent any accumulation of dust or other contaminants.

4.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

4.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the matrix being sampled. If interferences occur, cleanup may be necessary.

4.3 The extent of interferences that may be encountered using liquid chromatographic techniques has not been fully assessed. Although the HPLC conditions described allow for resolution of the specific compounds covered by this method, other matrix components may interfere.

4.4 Acetaldehyde is generated during the derivatization step due to the use of ethanol as the solvent for the DNPH. This background will impair the measurement of acetaldehyde at levels below 250 µg/L. Accordingly, if acetaldehyde is a compound of interest, use of another solvent, such as acetonitrile, for the DNPH solution is suggested.

## 5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method (2). A reference file of material safety data sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available.

5.2 Formaldehyde and acetaldehyde have been tentatively classified as known or suspected human or mammalian carcinogens.

## 6. EQUIPMENT AND SUPPLIES

6.1 SAMPLE CONTAINERS

6.1.1 Grab Sample Bottle (aqueous samples) -- 8 oz. (237 mL) amber glass, screw cap bottles and caps equipped with Teflon-faced silicone septa. Prior to use, wash bottles and septa.

6.1.2 Grab Sample Bottle (solids) -- 8 oz., amber glass, wide mouth, screw cap bottles and caps equipped with Teflon-lined closures. Prior to use wash bottles and septa.

6.2 REACTION VESSEL -- 250 mL Florence flask.

6.3 VIALS -- 10 and 25 mL, glass with Teflon-lined screw-caps.

6.4 VOLUMETRIC FLASKS -- 10 mL, with ground glass stopper.

6.5 BALANCE -- Analytical, capable of accurately weighing to the nearest 0.0001 g.

6.6 pH METER -- Capable of measuring to the nearest 0.01 units.

6.7 HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC APPARATUS (MODULAR)

6.7.1 Pumping System -- Gradient, with constant flow control capable of 1.50 mL/min.

6.7.2 High pressure injection valve with 20 µL loop.

6.7.3 Column -- 250 mm x 4.6 mm i.d., 5 µm particle size, C18 (Zorbax or equivalent).

6.7.4 Absorbance detector -- 360 nm.

6.7.5 Strip-chart recorder compatible with detector -- Use of a data system for measuring peak area and retention times is recommended.

6.8 LSE CARTRIDGES -- Packed with about 500 mg silica whose surface is modified by chemically bonded octadecyl (C-18) groups. These cartridges are available from several commercial suppliers. LSE disks may also be used as long as all the QC criteria specified in Sect. 9 of this method are met.

6.9 VACUUM MANIFOLD -- Capable of simultaneous extraction of 10 samples

6.10 SAMPLE RESERVOIRS -- 60-mL capacity.

6.11 PIPET -- Capable of accurately delivering 0.10 mL solution.

6.12 SYRINGES -- Luer-Lok, 5 mL, 500 µL and 100 µL.

6.13 ENVIRONMENTAL SHAKER -- Controlled temperature incubator (± 2°C) with orbital shaking (Lab-Line Orbit Environ-Shaker Model 3527 or equivalent).

## 7. REAGENTS AND STANDARDS

7.1 Reagent grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 REAGENT WATER -- All references to reagent water in this method refer to water in which an interference is not observed at the MDL of the compound of interest. Reagent water can be generated by passing tap water through a carbon filter bed containing about 1 lb. of activated carbon. Subsequently, while maintaining the temperature at 90°C, bubble a contaminant-free inert gas through the water for 1 hr. A water purification system may be used to generate organic-free deionized water.

7.3 METHANOL -- HPLC grade or equivalent.

7.4 ETHANOL -- Reagent grade

7.5 2,4- DINITROPHENYLHYDRAZINE (DNPH) (70% (W/W)) in water.

7.6 CITRIC ACID, $C_6H_8O_7$

7.7 SODIUM CITRATE -- Trisodium salt dihydrate.

7.8 SODIUM HYDROXIDE -- Concentrated

7.9 SODIUM CHLORIDE

7.10 SODIUM SULFITE, $Na_2SO_3$.

7.11 SODIUM SULFATE, $Na_2SO_4$ -- Granular, anhydrous.

7.12 HYDROCHLORIC ACID, 0.1 N.

7.13 ACETIC ACID -- Glacial.

7.14 AMMONIUM CHLORIDE, $NH_4Cl$.

7.15 EXTRACTION FLUID -- Dilute 64.3 mL of 1.0 N NaOH and 5.7 mL of glacial acetic acid to 900 mL with water. The pH should be 4.93 ± 0.02. Dilute to 1-L with water.

7.16 STOCK STANDARD SOLUTIONS

7.16.1 Stock standard formaldehyde solution approximately 1 mg/mL -- Prepare by diluting 265 µL of formalin to 100 mL with water.

7.16.2 Standardization of formaldehyde stock solution -- Transfer a 25-mL aliquot of a 0.1 M $Na_2SO_3$ solution to a beaker and record the pH. Add a 25.0 mL aliquot of the formaldehyde stock solution (Sect. 7.16.1) and record the pH. Titrate this mixture back to the original pH using 0.1 N HCl. The formaldehyde concentration is calculated using the following equation:

$$\text{Concentration (mg/mL)} = 30.03 \times (\text{N HCl}) \times (\text{mL HCl}) / 25.0$$

Where: N HCl = Normality of HCl solution used mL HCl = mL of standardized HCl solution used, and

30.03 = MW of formaldehyde

**Note:** The pH value of the 0.1 $Na_2SO_3$ should be 10.5 ± 0.2. When the stock formaldehyde solution and the 0.1 M $Na_2SO_3$ solution are mixed together as in Sect. 7.16.2, the pH should be 11.5 ± 0.2. It is recommended that new solutions be prepared if the pH deviates from this value.

7.16.3 Stock aldehyde(s) and ketone(s) -- Prepare by adding an appropriate amount of the analyte to 90 mL of methanol, then dilute to 100 mL to give a final concentration of 1.0 mg/mL.

7.16.4 Stock standard solutions must be replaced after 6 weeks, or sooner, if comparison with check standards indicates a problem.

7.17 REACTION SOLUTIONS

7.17.1 DNPH (3.00 g/L) -- Dissolve 428.7 mg of 70% (w/w) reagent in 100 mL absolute ethanol. Slight heating or sonication may be necessary to effect dissolution.

**Note:** If the DHPH does not complete by dissolve, filter the solution to remove the undissolved compound.

7.17.2 pH 3 Citrate buffer (1M) -- Prepare by adding 80 mL of 1 M citric acid solution to 20 mL 1 M sodium citrate solution. Mix thoroughly. Adjust pH with 6N NaOH or 6N HCl as needed.

7.17.3 Sodium chloride solution (saturated) -- Prepare by mixing an excess of the reagent grade solid with water.

7.17.4 Reducing Agent, Ammonium chloride (100 mg/L) -- Added to all samples containing residual chlorine. Sodium thiosulfate is not recommended because it may produce a residue of elemental sulfur that can interfere with some analytes. The ammonium chloride may be added as a solid with stirring until dissolved, to each volume of water.

7.18 SYRINGE FILTERS -- 0.45 µm filtration disks (Gelman Acrodisc No. 4438, or equivalent).

## 8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE COLLECTION -- When sampling from a water tap, open the tap and allow the system to flush until the water temperature has stabilized (usually about 2-5 min). Adjust the flow to about 500 mL/min and collect samples from the flowing stream. Keep samples sealed from collection time until analysis. When sampling from an open body of water, fill the sample container with water from a representative area. Sampling equipment must be free of plastic and other parts that may leach analytes into water. Follow other conventional sampling procedures (3) as needed.

8.2 SAMPLE DECHLORINATION AND PRESERVATION -- All samples should be iced or refrigerated at 4°C from the time of collection until extraction. Residual chlorine should be reduced at the sampling site by addition of a reducing agent (Sect. 7.17.4).

8.2.1 The use of HCL as a sample preservative has not been implemented in this method. Method 554 has been designed to measure "free" aldehydes and cyclohexanone by employing mild reaction conditions. The evolution of aldehydes can occur in samples whose pH is low (less than or equal to 2 (4), (5).

8.3 HOLDING TIME -- Samples must be derivatized and extracted within 3 days of sample collection. In reagent water, the analyte concentrations remained constant over a 7-day period. In ground water, hexanal, octanal and decanal experienced losses after the first day. The other analytes degraded after the third day. Matrices, such as groundwater, which are biologically active, should be extracted upon receipt. All samples should be extracted within three days of collection.

8.4 FIELD REAGENT BLANKS -- Processing of a field reagent blank (FRB) is recommended along with each sample set, which is composed of the samples collected from the same general sampling site at approximately the same time. At the laboratory, fill a sample container with reagent water, seal and ship to the sampling site along with the empty sample containers. Return the FRB to the laboratory with filled sample bottles.

## 9. QUALITY CONTROL

9.1 Each laboratory that uses this method is required to operate a formal QC program. Minimum QC requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified blanks, laboratory fortified sample matrices, and QC samples. Additional QC practices are encouraged.

9.2 LABORATORY REAGENTS BLANKS (LRB) -- Before processing any samples, the analyst must analyze at least one LRB to demonstrate the absence of contaminants that would prevent the determination of any method analyte. In addition, each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window of any analyte, the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

### 9.3 INITIAL DEMONSTRATION OF CAPABILITY

9.3.1 Select a representative fortified concentration for each of the target analytes at approximately 250 µg/L. Prepare a primary dilution standard (PDS) in methanol 1000 times more concentrated than the selected concentration. This primary dilution standard must be prepared independently from the standards used to prepare the calibration curve. With a syringe, add 100 µL of the PDS to each of four to seven 100 mL aliquots of reagent water. Analyze the aliquots according to the method beginning in Sect. 11.

9.3.2 Calculate the measured concentration of each analyte in each replicate, the mean concentration of each analyte in all replicates, the mean accuracy (as mean percentage of true value), the precision (RSD), and the MDL (1). Determine accuracy based upon extracted standards as called for in Sect. 10. For each analyte, the mean accuracy must fall in the range of R ± 30% using the values for reagent water listed in Table 3 at the lower concentration level. The calculated standard deviation should be less than ± 30% or 3 Sr (value listed in Table 3), whichever is larger. For those compounds that meet these criteria, performance is considered acceptable and sample analysis may begin. For those compounds that fail these criteria, the procedures in Sect. 9.3.1 must be repeated using a minimum of four fresh samples until satisfactory performance has been demonstrated.

9.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples using a new, unfamiliar method prior to obtaining some experience with it. As laboratory personnel gain experience with this method, the quality of data should improve beyond those required here.

9.3.4 The analyst is permitted to modify HPLC conditions. Each time a method modification is made, the analyst must repeat the procedures in Sect. 9.3.1.

### 9.4 LABORATORY FORTIFIED BLANK

9.4.1 The laboratory must analyze at least one laboratory fortified blank (LFB) sample with every 20 samples or one per sample set (all samples extracted within a 24-hr period), whichever frequency is greater. A fortified concentration near the lower value in Table 3 is recommended. The LFB sample must be prepared from a primary dilution standard which is prepared separately and independently from the standards used to prepare the calibration curve. Calculate the mean recovery (R). If the accuracy for any analyte falls outside the control limits (See Sect. 9.4.2), that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.4.2 Prepare control charts based on mean upper and lower control limits R ± 3 $S_r$. The initial demonstration of capability (Sect. 9.3) establishes the initial limits. After each 4 - 6 new accuracy measurements, recalculate R and $S_r$ using all the data, and construct new control limits. When the total number of data points reach 20, update the control limits by calculating R and $S_r$ using only the most recent 20 data points. At least quarterly, replicates of LFBs should be analyzed to determine the precision of the laboratory measurements. Add these results to the ongoing control charts to document data quality.

### 9.5 LABORATORY FORTIFIED SAMPLE MATRIX

9.5.1 The analyst must add known concentrations of analytes to a minimum of 10% of the routine samples or one concentration per sample set, whichever frequency is greater. The concentrations should be equal to or greater than the background concentrations in the sample selected for fortification. Ideally, the concentration should be the same as that used for the laboratory fortified blank (Sect. 9.4). Over time, samples from all routine sample sources should be fortified.

9.5.2 Calculate the mean percent recovery (R) for each analyte, after correcting the total mean measured concentration, A, from the fortified sample for the background concentration, B, measured in the unfortified sample, i.e.,

$$R = 100 (A - B) / C,$$

where C is the fortified concentration. Compare these values to control limits appropriate for reagent water data collected in the same fashion (Sect. 9.4).

9.5.3 If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Sect. 9.4), the accuracy problem encountered with the fortified sample is judged to be matrix related, not system related. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.6 QUALITY CONTROL SAMPLE (QCS) -- At least quarterly, analyze a QCS from an external source. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem source.

9.7 The laboratory may adopt additional QC practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements, or field reagent blanks may be used to assess contamination of samples under site, transportation and storage conditions.

## 10. CALIBRATION AND STANDARDIZATION

10.1 Establish HPLC operating parameters to completely separate the peaks. Table 1 lists some retention times produced using the following conditions:

HPLC Column: C18, 250 mm x 4.6 mm i.d., 5 $\mu$m particle size

| | |
|---|---|
| Mobile Phase: | 70%/30% methanol/water (v/v) for 20 min, up to 100% methanol in 15 min, hold at 100% methanol for 10 min |
| Flow Rate: | 1.5 mL/min |
| UV Detector: | 360 nm |
| Injection Size: | 20 µL |

10.2 Prepare procedural calibration standards according to the procedure in Sect. 10.2.1. Calibrate the chromatographic system using the external standard technique (Sect. 10.2.2).

10.2.1 Preparation of Calibration Standards

10.2.1.1 Prepare calibration solutions at a minimum of five concentration levels for each analyte of interest by adding volumes of stock standard solutions (Sect. 7.16) to reagent water and diluting to 100 mL. The lowest concentration level of each analyte should be near to, but above, the MDLs listed in Tables 1 or 2, while the other concentration levels should correspond to the expected range of concentrations found in real samples.

10.2.1.2 Process each calibration standard solution through derivatization and extraction using the same extraction option employed for sample processing (Sect. 11.1.3).

10.2.2 External standard calibration procedure

10.2.2.1 Analyze each processed calibration standard (some suggested chromatographic conditions are listed in Sect. 10.1) and tabulate peak area (y axis) versus calibration solution concentration (x axis) in µg/L. The results may be used to prepare calibration curves for the analytes. By linear regression, determine the slope, m, of the calibration curve.

10.2.2.2 The working calibration curve must be verified on each working day by the measurement of one or more fresh calibration standards. If the response for any analyte varies from the previously established responses by more than 10%, the test must be repeated after it is verified that the analytical system is in control. Alternatively, a new calibration curve may be prepared for that compound. If an autosampler is available, it may be convenient to prepare a calibration curve daily by analyzing standards along with test samples.

## 11. PROCEDURE

### 11.1 DERIVATIZATION AND EXTRACTION

11.1.1 Measure a 100-mL aliquot of the sample. Other sample volumes, from 50 mL to 100 mL, may be used to accommodate the anticipated analyte concentration range. Quantitatively transfer the sample aliquot to a 250 mL Florence flask.

**Note:** In cases where the selected sample or extract volume is less than 100 mL, the total volume of the aqueous phase should be adjusted to 100 mL with reagent water.

11.1.2 Derivatization and extraction of the derivatives can be accomplished using the liquid-solid extraction (Sect. 11.1.3).

11.1.3 Liquid-Solid Extraction (Either LSE cartridges or disks may be used.)

11.1.3.1 Add 4 mL of citrate buffer to the sample and adjust the pH to 3.0 ± 0.1 with 6 M HCl or 6 M NaOH. Add 6 mL of DNPH reagent, seal the container, and place in a heated, orbital shaker (Sect. 6.13), set at 40°C, for 1 hr. Adjust the agitation to gently swirl the reaction solution.

11.1.3.2 Assemble the vacuum manifold and connect it to a water aspirator or vacuum pump. Entrain three solid sorbent cartridges and attach the nested cartridges to the vacuum manifold. Condition the cartridges by passing 10 mL of dilute citrate buffer (10 mL of 1 M citrate buffer dissolved in 250 mL of water) through the cartridge train.

11.1.3.3 Remove the reaction vessel from the shaker and add 10 mL of saturated NaCl solution to the vessel.

11.1.3.4 Add the reaction solution to the cartridge train and apply a vacuum to draw the solution through the cartridges at a rate of 3 to 5 mL/min. Continue applying the vacuum for about 10 min after the liquid sample has passed through the cartridges.

11.1.3.5 While maintaining vacuum conditions, elute the cartridges with approximately 9 mL of absolute ethanol, directly into a 10-mL volumetric flask. Dilute the eluate to volume with absolute ethanol, mix thoroughly, and place in a tightly sealed vial until analysis.

**NOTE:** Because this method uses an excess of DNPH, the cartridges will retain a yellow color after this step. This color is not indicative of incomplete recovery of the analyte derivatives from the cartridges.

11.2 Analyze samples by HPLC, using recommended conditions provided in Sect. 10.1. Tables 1 and 2 list the retention times and MDLs that were obtained under these conditions. Other HPLC columns, chromatographic conditions, or detectors may be used provided the requirements of Sect. 9.3 are met.

11.3 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of the chromatograms.

11.4 If an analyte peak area exceeds the linear range of the calibration curve, a smaller sample volume should be used. Alternatively, the final solution may be diluted with ethanol and reanalyzed.

## 12. DATA ANALYSIS AND CALCULATIONS

12.1 Identify the method analytes in the sample chromatogram by comparing the retention time of the suspect peak to the retention time of an analyte peak in a calibration standard or the laboratory fortified blank. Computerized linear regression analysis is recommended for assessment of the linearity of the calibration and for slope calculation. Linearity is achieved when the coefficient of correlation (R) for the linear regression is $\geq 0.99$. Alternatively, the slope, m, may be determined by calculating the average peak area to concentration ratio for each calibration standard. The percent relative standard deviation (% RSD) of the slope must be less than 10% if the latter procedure is to be employed.

12.2 Calculate the analyte concentrations as follows:

$$C_s = \frac{A}{m} \times \frac{100}{V_s} \text{ (mL)}$$

where:

$m$ = slope of calibration curve, L/µg.
$C_s$ = sample concentration in µg/L;
$A$ = area of signal; and
$V_s$ = volume of sample in mL.

## 13. METHOD PERFORMANCE

13.1 The MDL concentrations listed in Tables 1 and 2 were obtained in reagent water and dechlorinated tap water using LSE. Results reported in both tables were achieved using fortified 100-mL samples.

13.2 This method has been tested for linearity of recovery from fortified reagent water and has been demonstrated to be applicable over the range from 10 x MDL to 1000 x MDL.

13.3 Single operator precision and accuracy data are provided in Tables 3, 4, 5 and 6. The tables report data at two fortification levels for reagent water, ground water and dechlorinated tap water. Data for ozone treated water are reported at a single fortification level (500 µg/L).

13.4 To generate the MDL and precision and accuracy data reported in this section, analytes were segregated into two fortification groups (A and B) and analyzed separately. Representative chromatograms using LSE for both Groups A and B are presented in Figures 1 and 2, respectively.

## 14. POLLUTION PREVENTION

14.1 This method utilizes the new liquid-solid extraction (LSE) technology to remove the analytes from water. It requires the use of very little organic solvent, thereby eliminating the hazards involved with the use of large volumes of organic solvents in conventional liquid-liquid extractions. A 10 mL aliquot of ethanol, a nontoxic solvent, is used per sample to elute the derivatized aldehydes and ketone from the LSE cartridge. This method is safe for the laboratory analyst to use and will not harm the environment.

## 15. WASTE MANAGEMENT

15.1 Due to the nature of this method and the reagents employed, there is no need for waste management in disposing of the used or unused samples. No toxic solvents or hazardous chemicals are used. The matrices are drinking water or source water and can be discarded in the sink drain.

## 16. REFERENCES

1. Glaser, J.A., D.L. Foerst, G.D. McKee, S.A. Quave, S.A., and W.L. Budde, "Trace Analyses for Wastewaters," _Environ_. _Sci_. _Technol_. 1981, 15, 1426-1435.

2. "OSHA Safety and Health Standards, General Industry," (29CRF1910). Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).

3. ASTM Annual Book of Standards, Part II, Volume 11.01, D3370-82, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, 1986.

4. "Aldehydes - Photometric Analysis," Sawicki, E and C.R. Sawicki, Volume 5; Academic Press, London: 1975.

5. Bicking, M.K.L., W.M. Cooke, F.K. Kawahara, and J.E. Longbottom, "Effect of pH on the Reaction of 2,4-Dinitrophenylhydrazine with Formaldehyde and Acetaldehyde," *J. of Chromatography*, 1988, 455, 310-315.

## 17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. METHOD DETECTION LIMITS[a] USING LIQUID-SOLID EXTRACTION IN REAGENT WATER

| Analyte | Retention Time (min) | MDL ($\mu$g/L) |
|---|---|---|
| Formaldehyde | 5.3 | 6.2 |
| Acetaldehyde | 7.5 | 43.7[b] |
| Propanal | 11.7 | 11.0 |
| Butanal | 18.1 | 6.3 |
| Pentanal | 26.9 | 15.3 |
| Hexanal | 32.5 | 10.7 |
| Heptanal | 36.6 | 10.0 |
| Octanal | 40.4 | 6.9 |
| Nonanal | 43.0 | 13.6 |
| Decanal | 45.5 | 4.4 |
| Cyclohexanone | 27.9 | 5.8 |
| Crotonaldehyde | 16.7 | 7.7 |

[a] MDL was computed as follows: MDL = t(N-1, 0.01) x S.D.

where t(n-1, 0.01) is the upper first percentile point of the t-distribution with n-1 degrees of freedom and S.D. is the standard deviation in $\mu$g/L. With the exception of acetaldehyde all reported MDLs are based upon analyses of 6 to 8 replicate, fortified blanks (25 $\mu$g/L). (See Reference 1)

[b] Reported MDL based upon analyses of 3 replicate, fortified blanks at 250 $\mu$g/L.

## TABLE 2. METHOD DETECTION LIMITS[a] USING LIQUID-SOLID EXTRACTION IN DECHLORINATED TAP WATER

| Analyte | Retention Time (min) | MDL ($\mu$g/L) |
|---|---|---|
| Formaldehyde | 5.3 | 8.1 |
| Acetaldehyde | 7.5 | 69.0[b] |
| Propanal | 11.7 | 3.4 |
| Butanal | 18.1 | 8.6 |
| Pentanal | 26.9 | 3.3 |
| Hexanal | 32.5 | 9.6 |
| Heptanal | 36.6 | 7.3 |
| Octanal | 40.4 | 6.0 |
| Nonanal | 43.0 | 24.3 |
| Decanal | 45.5 | 12.9 |
| Cyclohexanone | 27.9 | 9.5 |
| Crotonaldehyde | 16.7 | 6.3 |

---

[a] MDL was computed as follows: MDL = t(N-1, 0.01) x S.D.

where t(n-1, 0.01) is the upper first percentile point of the t-distribution with n-1 degrees of freedom and S.D. is the standard deviation in $\mu$g/L. With the exception of acetaldehyde all reported MDLs are based upon analyses of 6 to 8 replicate, fortified blanks (25 $\mu$g/L). (See Reference 1)

[b] Reported MDL based upon analyses of 3 replicate, fortified blanks at 250 $\mu$g/L.

## TABLE 3. SINGLE OPERATOR ACCURACY AND PRECISION USING LIQUID-SOLID EXTRACTION IN REAGENT WATER

| Analyte | FL[a] | R[b] | Sr[c] | Number of Analyses |
|---|---|---|---|---|
| Formaldehyde | 250 | 96.3 | 7.6 | 7 |
| | 2500 | 109.8 | 1.5 | 3 |
| Acetaldehyde | 250 | 40.2 | 1.0 | 3 |
| | 2500 | 112.2 | 21.3 | 3 |
| Propanal | 250 | 93.8 | 2.3 | 7 |
| | 2500 | 110.8 | 2.4 | 3 |
| Butanal | 250 | 91.1 | 2.9 | 7 |
| | 2500 | 108.2 | 2.6 | 3 |
| Pentanal | 250 | 91.6 | .20 | 7 |
| | 2500 | 100.5 | 2.0 | 3 |
| Hexanal | 250 | 87.0 | 4.7 | 7 |
| | 2500 | 94.6 | 5.4 | 3 |
| Heptanal | 250 | 90.1 | 2.4 | 7 |
| | 2500 | 104.9 | 1.7 | 3 |
| Octanal | 250 | 89.2 | .09 | 7 |
| | 2500 | 97.1 | 1.0 | 3 |
| Nonanal | 250 | 90.2 | 3.0 | 7 |
| | 2500 | 105.3 | 2.2 | 3 |
| Decanal | 250 | 85.0 | 1.1 | 7 |
| | 2500 | 98.9 | 1.6 | 3 |
| Crotonaldehyde | 250 | 87.6 | 7.3 | 7 |
| | 2500 | 104.3 | 1.5 | 3 |
| Cyclohexanone | 250 | 94.8 | 4.1 | 7 |
| | 2500 | 116.7 | 4.7 | 3 |

[a] FL = Fortification Level in μg/L

[b] R = Average Percent Recovery

[c] Sr = Standard Deviation of Percent Recovery

## TABLE 4. SINGLE OPERATOR ACCURACY AND PRECISION USING LIQUID-SOLID EXTRACTION IN GROUND WATER

| Analyte | FL[a] | R[b] | Sr[c] | Number of Analyses |
|---|---|---|---|---|
| Formaldehyde | 250 | 103.2 | 10.3 | 7 |
| | 2500 | 118.4 | 9.2 | 3 |
| Acetaldehyde | 250 | 50.2 | 3.9 | 3 |
| | 2500 | 109.2 | 6.5 | 3 |
| Propanal | 250 | 99.1 | 1.3 | 7 |
| | 2500 | 105.4 | 9.4 | 3 |
| Butanal | 250 | 94.7 | 3.9 | 7 |
| | 2500 | 95.9 | 8.7 | 3 |
| Pentanal | 250 | 90.0 | 12.7 | 3 |
| | 2500 | 96.7 | 1.6 | 3 |
| Hexanal | 250 | 89.0 | 4.7 | 6 |
| | 2500 | 95.9 | 1.9 | 3 |
| Heptanal | 250 | 96.4 | 6.3 | 7 |
| | 2500 | 98.0 | 9.6 | 3 |
| Octanal | 250 | 94.1 | 1.8 | 7 |
| | 2500 | 96.9 | 1.5 | 3 |
| Nonanal | 250 | 93.1 | 5.1 | 7 |
| | 2500 | 97.9 | 11.6 | 3 |
| Decanal | 250 | 86.0 | 4.3 | 7 |
| | 2500 | 98.5 | 2.2 | 3 |
| Crotonaldehyde | 250 | 93.6 | 3.0 | 7 |
| | 2500 | 100.2 | 2.6 | 3 |
| Cyclohexanone | 250 | 107.6 | 4.0 | 7 |
| | 2500 | 111.1 | 10.8 | 3 |

[a] FL = Fortification Level in µg/L

[b] R = Average Percent Recovery

[c] Sr = Standard Deviation of Percent Recovery

## TABLE 5. SINGLE OPERATOR ACCURACY AND PRECISION USING LIQUID-SOLID EXTRACTION IN DECHLORINATED TAP WATER

| Analyte | FL[a] | R[b] | Sr[c] | Number of Analyses |
|---|---|---|---|---|
| Formaldehyde | 25 | 90.0 | 1.1 | 3 |
| | 250 | 90.8 | 11.6 | 8 |
| Acetaldehyde | 250[d] | 52.0 | 9.7 | 8 |
| Propanal | 25 | 120.2 | 17.9 | 8 |
| | 250 | 83.4 | 6.3 | 8 |
| Butanal | 25 | 91.6 | 11.4 | 8 |
| | 250 | 79.4 | .81 | 3 |
| Pentanal | 25 | 106.1 | 1.1 | 8 |
| | 250 | 72.3 | 3.9 | 3 |
| Hexanal | 25 | 99.2 | 3.2 | 8 |
| | 250 | 70.4 | 4.0 | 3 |
| Heptanal | 25 | 97.2 | 9.8 | 8 |
| | 250 | 79.2 | 5.4 | 8 |
| Octanal | 25 | 60.5 | 2.0 | 8 |
| | 250 | 75.6 | 14.8 | 3 |
| Nonanal | 25 | 120.6 | 32.4 | 8 |
| | 250 | 69.9 | 12.7 | 3 |
| Decanal | 25 | 109.8 | 23.9 | 8 |
| | 250 | 91.5 | 34.2 | 3 |
| Crotonaldehyde | 25 | 86.8 | 2.1 | 8 |
| | 250 | 97.7 | 5.5 | 8 |
| Cyclohexanone | 25 | 94.8 | 12.7 | 8 |
| | 250 | 104.5 | 10.8 | 8 |

[a] FL = Fortification Level in μg/L

[b] R = Average Percent Recovery

[c] Sr = Standard Deviation of Percent Recovery

[d] Background levels of this analyte will impair measurement if the fortification level is below 250 μg/L.

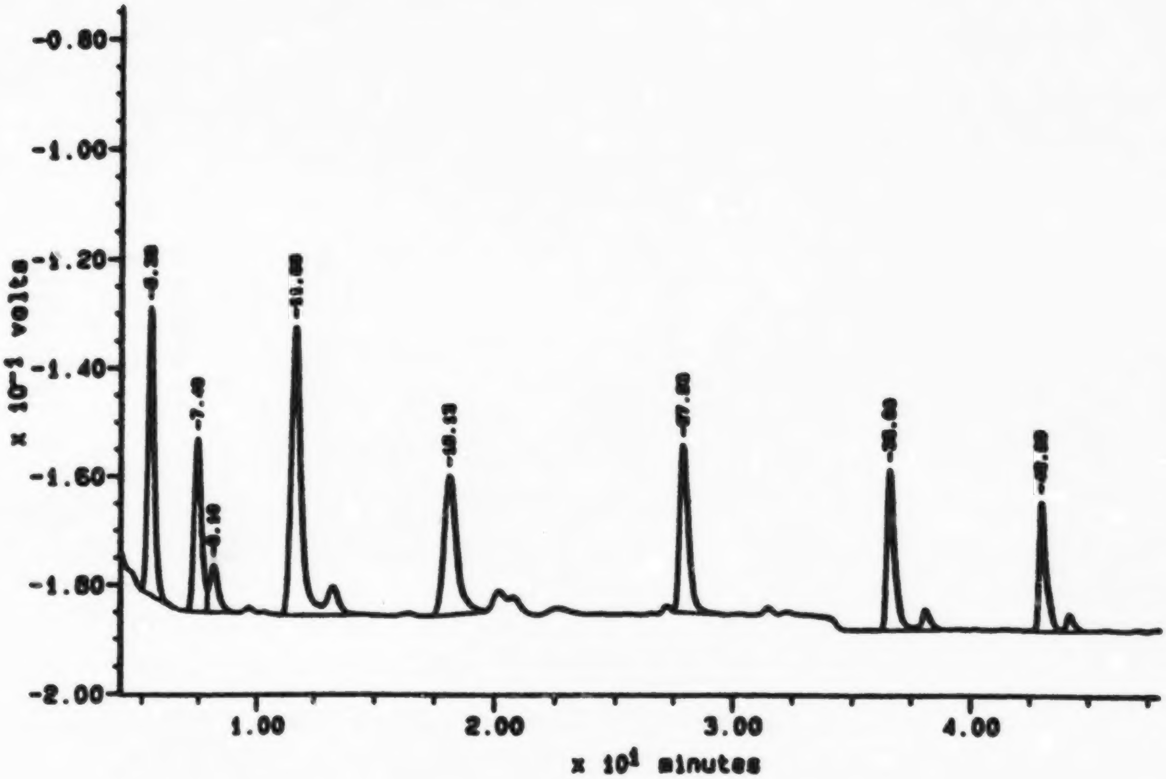
## TABLE 6. SINGLE OPERATOR ACCURACY AND PRECISION USING LIQUID-SOLID EXTRACTION IN OZONE TREATED WATER

| Analyte | FL[a] | R[b] | Sr[c] | Number of Analyses |
|---|---|---|---|---|
| Formaldehyde | 500 | 78.8 | 1.1 | 8 |
| Acetaldehyde | 500 | 99.4 | 2.3 | 8 |
| Propanal | 500 | 93.7 | 1.8 | 8 |
| Butanal | 500 | 97.6 | 1.2 | 8 |
| Pentanal | 500 | 89.6 | 1.7 | 8 |
| Hexanal | 500 | 91.8 | 1.7 | 8 |
| Heptanal | 500 | 99.0 | 3.2 | 8 |
| Octanal | 500 | 93.9 | 2.5 | 8 |
| Nonanal | 500 | 100.0 | 3.8 | 8 |
| Decanal | 500 | 93.4 | 6.9 | 8 |
| Crotonaldehyde | 500 | 89.5 | 2.5 | 8 |
| Cyclohexanone | 500 | 97.2 | .83 | 8 |

[a] FL = Fortification Level in µg/L

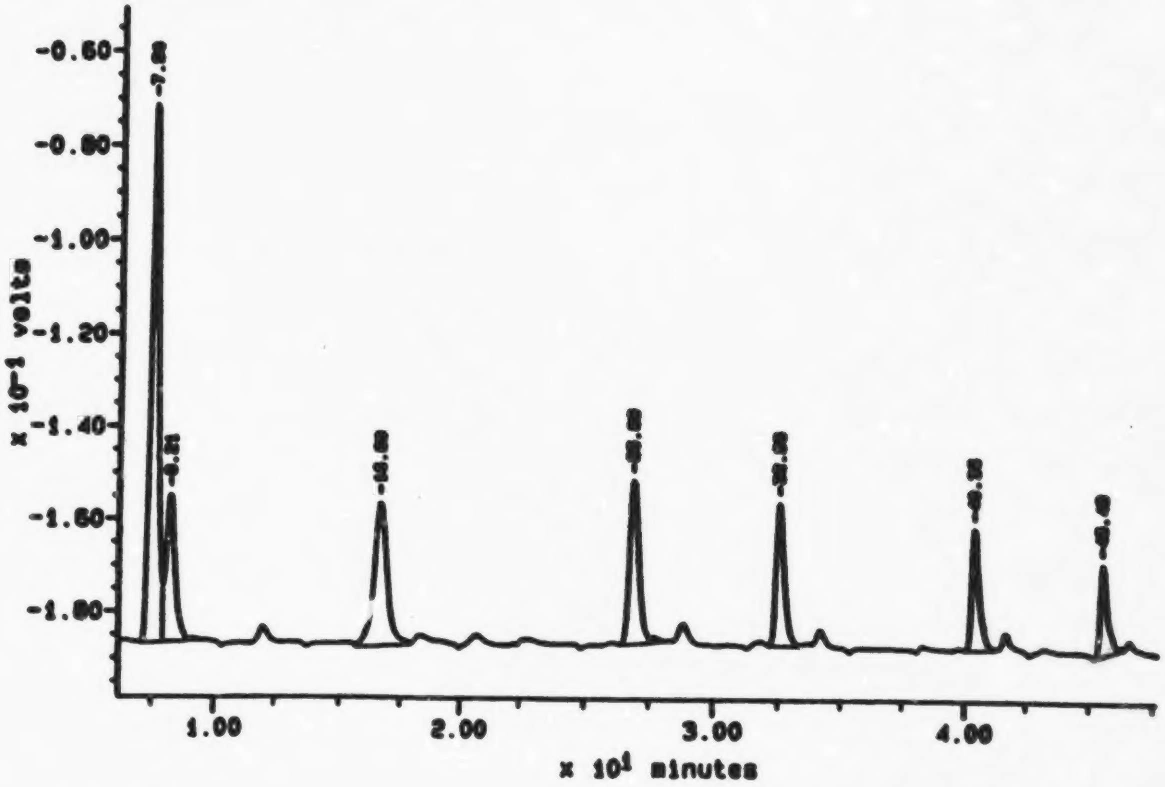
[b] R = Average Percent Recovery

[c] Sr = Standard Deviation of Percent Recovery



| RT (min) | Analyte |
|---|---|
| 5.33 | Formaldehyde |
| 11.68 | Propanal |
| 18.13 | Butanal |
| 27.93 | Cyclohexanone |
| 36.60 | Heptanal |
| 42.99 | Nonanal |

Figure 1 Liquid-solid Procedural Standard of Group A Analytes at 625 µg/L.



| RT (min) | Analyte |
|---|---|
| 7.50 | Acetaldehyde |
| 16.68 | Crotonaldehyde |
| 26.88 | Pentanal |
| 32.53 | Hexanal |
| 40.36 | Octanal |
| 45.49 | Decanal |

Figure 2 Liquid-solid Procedural Standard of Group B Analytes at 625 µg/L.

# METHOD 555. DETERMINATION OF CHLORINATED ACIDS IN WATER BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH A PHOTODIODE ARRAY ULTRAVIOLET DETECTOR

Revision 1.0

August 1992

James W. Eichelberger

Winslow J. Bashe (Technology Applications, Inc.)

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U. S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

# METHOD 555

## DETERMINATION OF CHLORINATED ACIDS IN WATER BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH A PHOTODIODE ARRAY ULTRAVIOLET DETECTOR

### 1. SCOPE AND APPLICATION

1.1 This is a high performance liquid chromatographic (HPLC) method for the determination of certain chlorinated acids in ground water and finished drinking water. The following compounds can be determined by this method:

| Analyte | Chemical Abstract Services Registry Number |
|---|---|
| Acifluorfen | 50594-66-6 |
| Bentazon | 25057-89-0 |
| Chloramben[a] | 133-90-4 |
| 2,4-D | 94-75-7 |
| 2,4-DB | 94-82-6 |
| Dicamba | 1918-00-9 |
| 3,5-Dichlorobenzoic acid | 51-36-5 |
| Dichlorprop | 120-36-5 |
| Dinoseb | 88-85-7 |
| 5-Hydroxydicamba[a] | 7600-50-2 |
| MCPA | |
| MCPP | |
| 4-Nitrophenol[a] | 100-02-7 |
| Pentachlorophenol[b] (PCP) | 87-86-5 |
| Picloram[a] | 1918-02-1 |
| 2,4,5-T | 93-76-5 |
| 2,4,5-TP | 93-72-1 |

[a] Analytes measurable from 20 mL sample volume only.

[b] Use a 100 mL sample for pentachlorophenol in order to attain a MDL of 0.3 µg/L. The MLC for this compound is 1.0 µg/L.

1.2 This method is applicable to the determination of salts and esters of analyte acids. The form of each analyte is not distinguished by this method. Results are calculated and reported for each listed analyte as the total free acid.

1.3 This method has been validated in a single laboratory and method detection limits (MDLs) (1) have been determined from a 20-mL sample for the analytes above. Observed MDLs may vary among ground waters, depending on the nature of interferences in the sample matrix and the specific instrumentation used.

1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC and in the interpretation of chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Sect. 9.3.

1.5 Analytes that are not separated chromatographically cannot be individually identified and measured in the same calibration mixture or water sample unless an alternative technique for identification and quantitation exists (Sect. 11.3).

1.6 When this method is used to analyze unfamiliar samples, analyte identifications must be confirmed by at least one additional qualitative technique.

## 2. SUMMARY OF METHOD

2.1 A measured sample volume of approximately 100 mL is adjusted to pH 12 with 6 N sodium hydroxide, shaken, and allowed to set for 1 hr to hydrolyze chlorinated esters. The sample is acidified with $H_3PO_4$, filtered, and the chlorinated acids are extracted from a 20-mL aliquot. The 20-mL aliquot is pumped through an HPLC cartridge (containing $C_{18}$-silica), trapping the chlorinated acids. The concentrator cartridge is valved in-line with the $C_{18}$ analytical column following extraction. The analytes are separated and measured by photodiode array - ultraviolet detection (PDA-UV). NOTE: A liquid-solid extraction disk is perfectly acceptable for use in the in-line extraction of the analytes providing all quality control (QC) criteria in Sect. 9 are met or exceeded.

2.2 The method measures the analytes from 20-mL volumes. Volumes of up to 100 mL may be analyzed by this procedure for certain analytes. The analytes which may **not** be determined in a larger volume are indicated in Sect. 1.1.

## 3. DEFINITIONS

3.1 LABORATORY DUPLICATES (LD1 AND LD2) -- Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.2 FIELD DUPLICATES (FD1 AND FD2) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.3 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.4 FIELD REAGENT BLANK (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.5 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.6 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- An aliquot of an environmental sample to which know quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.7 STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial supplier.

3.8 PRIMARY DILUTION STANDARD SOLUTION (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.9 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.10 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards, It is used to check laboratory performance with externally prepared test materials.

3.11 METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99%

confidence that the analyte concentration is greater than zero.

3.12 EXTERNAL STANDARD (ES) -- A pure analyte(s) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the external standard(s) is used to calibrate the instrument response for the corresponding analytes(s). The instrument response is used to calculate the concentrations of the analyte(s) in the sample.

## 4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in liquid chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by analyzing laboratory reagent blanks as described in Sect. 9.2.

4.1.1 Glassware must be scrupulously cleaned (3). Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with dilute acid, tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 1 hr. Do not heat volumetric ware. Thermally stable materials such as PCBs might not be eliminated by this treatment. Thorough rinsing with acetone may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

4.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required. WARNING: When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous. Removal of preservatives by distillation may also reduce the shelf-life of the solvent.

4.2 The acid forms of the analytes are strong organic acids which react readily with alkaline substances and can be lost during sample preparation. Glassware must be acid-rinsed with 1 N hydrochloric acid prior to use to avoid analyte losses due to adsorption.

4.3 Matrix interferences may be caused by contaminants that are coextracted from the sample. Also, note that all method analytes are not resolved from each other on a single column, i.e., one analyte of interest may interfere with another analyte of interest. The extent of matrix interferences will vary considerably from source to source, depending upon the water sampled. The procedures in Sect. 11 can be used to overcome many of these interferences. Tentative identifications should always be confirmed (Sect. 11.3).

## 5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. Accordingly, exposure to these chemicals must be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets should also be made available to all personnel involved in the chemical analysis.

5.2 WARNING: When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous. Therefore, storage of large volumes of purified solvents may be hazardous. Therefore, only small volumes of solvents should be purified just before use.

## 6. EQUIPMENT AND SUPPLIES

6.1 SAMPLE BOTTLE -- Borosilicate, 125-mL volume, graduated, fitted with Teflon-lined screw cap. Protect samples from light. The container must be washed and dried as described in Sect. 4.1.1 before use to minimize contamination. Cap liners may be cut to fit from Teflon sheets and extracted with methanol overnight prior to use.

6.2 GLASSWARE

6.2.1 Volumetric flask, Class A -- 100 mL, with ground glass stoppers.

6.2.2 Graduated cylinder -- 100 mL

6.2.3 Disposable pipets, Transfer -- borosilicate glass

6.2.4 Glass syringe -- 50 mL, with Luer-Lok fitting

6.2.5 Volumetric pipette, Class A -- 20 mL

6.3 BALANCE -- Analytical, capable of accurately weighing to the nearest 0.0001 g.

6.4 LIQUID CHROMATOGRAPH -- Analytical system complete with gradient programmable HPLC suitable for use with analytical HPLC columns and all required accessories including an injector, analytical column, semi-prep guard column, and photodiode array UV detector. A data system is necessary for measuring the peak areas and for assessing the confirmation of the peak identification. A personal computer (PC) of at least the AT-class is generally needed to control and collect data from the photodiode array UV detector. Table 1 lists the retention times observed for the method analytes using the column and analytical conditions described below. Figure 1 is a schematic drawing of the analytical system including the sample concentrator column (semi-prep guard column).

6.4.1 Primary Column -- 250 mm x 4.6 mm I.D. ODS-AQ, 5 µm spherical (YMC Ltd.). Any column may be used if equivalent or better performance (better peak shape, better analyte efficiency, or more complete separation of analytes) can be demonstrated. Mobile phase flow rate is established at 1.0 mL/min (linear velocity of 6.0 cm/min). Two mobile phase components are used: A -- 0.025 M $H_3PO_4$; B -- Acetonitrile. A gradient solvent program is used to separate the analytes: 90:10 A:B to 10:90 A:B in 30 min, linear ramp / hold at 10:90 for 10 min. Reverse the gradient and establish initial conditions: 10:90 A:B to 90:10 A:B in 10 min, linear ramp. Allow column backpressure to restablize for 5 to 10 min before beginning the next analysis. Total restabilization time will be determined by each analyst.

6.4.2 Confirmation Column -- 300 mm x 3.9 mm I.D. Nova-Pak $C_{18}$, 4 µm spherical (Waters Chromatography Division, Millipore). Any column may be used if equivalent or better performance (better peak shape, better analyte efficiency, or more complete separation of analytes) can be demonstrated. Mobile phase and conditions same as primary column.

6.4.3 Sample Concentrator Column -- 30 mm x 10 mm I.D. ODS-AQ, 5 µm spherical (YMC Ltd). An alternative concentrator column may be used if all QC criteria in Sect. 9 can be equalled or improved. Also, a liquid-solid extraction disk may be used if all QC criteria in Sect. 9 can be equalled or improved.

6.4.4 6-port Switching Valve -- Rheodyne Model 7000 (Rheodyne Corp).

6.4.5 Sample Delivery Pump -- A piston-driven pump capable of delivering aqueous sample at a flow rate of 5.0 mL/min. An analytical HPLC pump may serve as the sample delivery pump. A Waters Model 6000A was used to generate the data presented in this method.

6.4.6 Detector -- Photodiode Array - Ultraviolet (PDA-UV), LKB-Bromma Model 2140 Rapid Spectral Detector or equivalent. Detector parameters: Scan Range - 210 to 310 nm at 1 scan/sec, detector integration - 1 sec.

6.4.7 Data Handling System -- DOS-based Personal Computer, AT-class machine or machine of greater capability with 640 K RAM or more, an 80 Mb hard disk or larger, VGA monitor or equivalent.

## 7. REAGENTS AND STANDARDS

7.1 ACETONITRILE -- HPLC Grade or equivalent.

7.2 SODIUM SULFITE, GRANULAR, ANHYDROUS -- ACS Grade.

7.3 SODIUM HYDROXIDE (NAOH), PELLETS -- ACS Grade.

7.3.1 NaOH, 6 N -- Dissolve 216 g NaOH in 900 mL reagent water.

7.4 PHOSPHORIC ACID, 85% AR, -- ACS grade.

7.4.1 0.025 M -- Mix 2.0 mL of $H_3PO_4$ in 998 mL of reagent water.

7.5 STOCK STANDARD SOLUTIONS (1.00 $\mu$g/$\mu$L) -- Stock standard solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedure:

7.5.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in acetonitrile and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

7.5.2 Transfer the stock standard solutions into Teflon-lined sealed screw cap amber vials. Store at room temperature and protect from light.

7.5.3 Stock standard solutions should be replaced after two months or sooner if comparison with laboratory fortified blanks, or QC samples indicate a problem.

7.6 HYDROCHLORIC ACID -- ACS grade.

7.6.1 HCl, 1 N -- Dilute 50 mL in 600 mL of reagent water.

7.7 Filters, 0.45 $\mu$m, Nylon, 25 mm i.d. (Gelman Sciences)

## 8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Grab samples must be collected in glass containers. Conventional sampling practices (2) should be followed; however, the bottle must not be prerinsed with sample before collection.

8.2 SAMPLE PRESERVATION AND STORAGE

8.2.1 Add hydrochloric acid (1:1) to the sample to produce a pH of 2. The pH may be measured in the field using pH indicator strips.

8.2.2 Residual chlorine should be reduced at the sampling site by the addition of a reducing agent. Add 4-5 mg of sodium sulfite (this may be added as a solid with shaking until dissolved) to each 100 mL of water.

8.2.3 The samples must be iced or refrigerated at 4°C away from light from the time of collection until extraction. The samples must be analyzed within 14 days of collection. However, analyte stability may be affected by the matrix. Therefore, the analyst should verify that the preservation technique is applicable to the samples under study. If the 14-day holding time is exceeded, the data should be flagged so that the data user is aware of possible analyte degradation.

8.2.4 Field reagent blanks (FRB) -- Processing a (FRB) is recommended along with each set, which is composed of the samples collected from the same general sample site at approximately the same time. At the laboratory, fill a sample container with reagent water, seal, and ship to the sampling site along with the empty sample containers. During sample collection, open the FRB and add H6 (Sect. 8.2.1) and sodium sulfite (Sect. 8.2.2) Return the FRB to the laboratory with filled sample bottles.

## 9. QUALITY CONTROL

9.1 Minimum QC requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified samples, laboratory fortified blanks, and QC samples.

9.2 LABORATORY REAGENT BLANKS (LRB) -- Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window of any analyte the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

### 9.3 INITIAL DEMONSTRATION OF CAPABILITY

9.3.1 Select a representative fortified concentration for each analyte. Prepare a sample concentrate (in acetonitrile) containing each analyte at 1000 times the selected concentration. With a syringe, add 100 µL of the concentrate to each of at least four 100-mL aliquots of reagent water, and analyze each aliquot according to procedures beginning in Sect. 11.

9.3.2 Calculate the recoveries, the relative standard deviation, and the MDLs (5). For each analyte the recovery value for all four of these samples must fall in the range of R ± 30%, using the value for R for reagent water in Table 2. As the calibration procedure employs a fortified reagent water blank for the determination of the calibration curves or factors, the recovery values for the analytes should, by definition, be within this range. If the mean recovery of any analyte fails this demonstration, repeat the measurement of that analyte to demonstrate acceptable performance.

9.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples using a new, unfamiliar method prior to obtaining some experience with it. As laboratory personnel gain experience with this method the quality of data should improve beyond what is required here.

9.4 The analyst is permitted to modify LC columns, LC conditions, and detectors. Each time such method modifications are made, the analyst must repeat the procedures in Sect. 9.3. NOTE: The LC column and guard cartridge used to generate the data in this method were found to be unique $C_{18}$-silica columns. Before substituting other $C_{18}$ columns, a careful review of the literature is recommended.

9.5 ASSESSING LABORATORY PERFORMANCE -- Laboratory fortified blank

9.5.1 The laboratory must analyze at least one laboratory fortified blank (LFB) sample with every 20 samples or one per sample set (all samples analyzed within a 24-hr period) whichever is greater. The concentration of each analyte in the LFB should be 10 times the MDL or the MCL, whichever is less. Calculate accuracy as percent recovery ($X_i$). If the recovery of any analyte falls outside the control limits (See Sect. 9.5.2), that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.5.2 Until sufficient data become available from within the laboratory, usually a minimum of results from 20 to 30 analyses, the laboratory should assess laboratory performance against the control limits in Sect. 9.3.2 that are derived from the data in Table 2. When sufficient internal performance data become available, develop control limits from the mean percent recovery (X) and standard deviation (S) of the percent recovery. These data are used to establish upper and lower control limits as follows:

UPPER CONTROL LIMIT = X + 3S
LOWER CONTROL LIMIT = X - 3S

After each five to ten new recovery measurements, new control limits should be calculated using only the most recent 20-30 data points. These calculated control limits should never exceed those established in Sect. 9.3.2.

9.5.3 It is recommended that the laboratory periodically determine and document its detection limit capabilities for the analytes of interest.

9.5.4 At least quarterly, analyze a QC sample from an outside source.

9.5.5 Laboratories are encouraged to participate in external performance evaluation studies such as the laboratory certification programs offered by many states or the studies conducted by USEPA. Performance evaluation studies serve as independent checks on the analyst's performance.

### 9.6 ASSESSING ANALYTE RECOVERY -- Laboratory fortified sample matrix

9.6.1 The laboratory should add a known concentration to a minimum of 10% of the routine samples or one sample per set, whichever is greater. The concentration should not be less than the background concentration of the sample selected for fortification. Ideally, the concentration should be the same as that used for the laboratory fortified blank (Sect. 9.5). Over time, samples from all routine sample sources should be fortified.

9.6.2 Calculate the percent recovery, P of the concentration for each analyte, after correcting the analytical result, X, from the fortified sample for the background concentration, b, measured in the unfortified sample,

$$P = 100 \, (X - b) \, / \text{ fortifying concentration},$$

and compare these values to control limits appropriate for reagent water data collected in the same fashion. If the analyzed unfortified sample is found to contain **NO** background concentrations, and the added concentrations are those specified in Sect. 9.5, the appropriate control limits would be the acceptance limits in Sect. 9.5. If, on the other hand, the analyzed unfortified sample is found to contain background concentration, b, estimate the standard deviation at the background data, $s_b$, using regressions or comparable background data and similarly, estimate the mean, $X_a$, and standard deviation, $s_a$, of analytical results or the total concentration after fortifying. Then the appropriate percentage control limits would be P ± $3s_p$, where:

$$P = 100 \, X \, / \, (b + \text{fortifying concentration})$$

$$\text{and } s_p = 100 \, (s_a^2 + s_b^2)^{1/2} \, / \text{fortifying concentration}$$

For example, if the background concentration for Analyte A was found to be 1 µg/L and the added amount was also 1 µg/L, and upon analysis the laboratory fortified sample measured

1.6 µg/L, then the calculated P for this sample would (1.6 µg/L minus 1.0 µg/L)/ 1.0 µg/L or 60%. This calculated P is compared to control limits derived from prior reagent water data. Assume it is known that analysis of an interference free sample at 1.0 µg/L yields an s of 0.12 µg/L and similar analysis at 2.0 µg/L yields X and S of 2.01 µg/L and 0.20 µg/L, respectively. The appropriate limits to judge the reasonableness of the percent recovery, 60%, obtained on the fortified matrix sample is computed as follows:

$$[100\ (2.01\ \mu g/L)\ /\ 2.0\ \mu g/L]$$
$$\pm\ 3\ (100)\ [(0.12\ \mu g/L)^2 + (0.20\ \mu g/L)^2]^{1/2}\ /\ 1.0\ \mu g/L =$$
$$100.5\%\ \pm\ 300\ (0.233) =$$

100.5% ± 70 or 30% to 170% recovery of the added analyte.

9.6.3 If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Sect. 9.5), the recovery problem encountered with the fortified sample is judged to be matrix related, not system related. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.7 The laboratory may adopt additional QC practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements. The field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage.

## 10. CALIBRATION AND STANDARDIZATION

10.1 Establish HPLC operating parameters equivalent to those indicated in Sect. 6.4.1. The HPLC system should be calibrated using the external standard technique (Sect. 10.2). NOTE: Calibration standard solutions must be prepared such that no unresolved analytes are mixed together. The method analytes have been separated into two calibration solutions (See Table 1 for Groups A and B). The analytes in these solutions have been found to be resolved under the LC conditions listed. Mixtures of these analytes at concentration levels of 100 µg/mL (in acetonitrile) are suggested as a possible secondary dilution standard. Figures 2 and 3 are typical chromatograms of Groups A and B as separated on the primary HPLC column.

### 10.2 EXTERNAL STANDARD CALIBRATION PROCEDURE

10.2.1 Prepare calibration standards (CAL) at a minimum of three (five are recommended) concentration levels for each analyte of interest by adding volumes of one or more stock standards to volumetric flasks. Alternatively, add various volumes of a primary dilution standard solution of Group A or B (Sect. 10.1) to a volumetric flask. Dilute to volume with the aqueous mobile phase (0.025 M $H_3PO_4$). The lowest standard should contain analyte concentrations near, but above, the respective MDL. The remaining standards should bracket the analyte concentrations expected in the sample extracts, or should define the working range of the detector.

10.2.2 Starting with the standard of the lowest concentration, process each calibration standard according to Sect. 11.1 and tabulate response (peak area) versus injected quantity in the standard. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to concentration (response factor) is a constant over the working range (20% RSD or less), linearity through the origin can be assumed and the average ratio or response factor can be used in place of a calibration curve.

10.2.3 The working calibration curve or response factor must be verified on each working day by the measurement of a CAL, analyzed at the beginning of the analysis day. It is highly recommended that an additional check standard be analyzed at the end of the analysis day. For extended periods of analysis (greater than 8 hr), it is strongly recommended that check standards be interspersed with samples at regular intervals during analyses. If the response for any analyte varies from the predicted response by more than ± 25%, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a new calibration curve.

10.2.4 Verify calibration standards periodically, recommend at least quarterly, by analyzing a standard prepared from reference material obtained from an independent source. Results from these analyses must be within the limits used to routinely check calibration.

## 11. PROCEDURE

### 11.1 HYDROLYSIS, PREPARATION, AND EXTRACTION.

11.1.1 Add preservative to blanks and QC check standards. Mark the water meniscus on the side of the sample bottle for later determination of sample volume (Sect. 11.1.5).

11.1.2 Add 1.7 mL of 6 N NaOH to the sample, seal, and shake. Check the pH of the sample with pH paper; if the sample does not have a pH greater than or equal to 12, adjust the pH by adding more 6 N NaOH. Let the sample sit at room temperature for 1 hr, shaking the sample bottle and contents periodically.

11.1.3 Add 2 mL of concentrated $H_3PO_4$ to the sample, seal, and shake to mix. Check the pH of the sample with pH paper; if the sample does not have a pH less than or equal to two, adjust the pH by adding more $H_3PO_4$.

11.1.4 From the homogeneous sample, remove a 20-mL aliquot for analysis. Filter the aliquot through a 0.45 µm filter into a graduated cylinder or other convenient graduated container. Using an HPLC pump (or HPLC reagent delivery pump), pump the 20-mL aliquot through the on-line concentrator column at a flowrate of 5.0 mL/min (See Figure 1). The use of a liquid-solid extraction disk is perfectly acceptable providing all QC criteria in Sect. 9 are met or exceeded. After passing the sample through the concentrator column, follow with an additional 10-mL of the aqueous mobile phase (0.025 M $H_3PO_4$).

11.1.5 After analysis is completed, determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 100-mL graduated cylinder. Record the sample volume to the nearest 1 mL.

## 11.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

11.2.1 Sect. 6.4.1 summarizes the recommended operating conditions for the HPLC. Included in Table 1 are retention times observed using this method. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of Sect. 9.3 are met.

11.2.2 Calibrate the system daily as described in Sect. 10.

11.2.3 After loading the sample (or calibration standard) onto the concentrator column, valve the sample into the analytical stream, backflushing the concentrator column. The photodiode array detector (PDA-UV) is set to scan and record from 210 to 310 nm, 1 scan per second during the entire chromatographic run (40 min). Extract the 230 nm trace from the stored data and record the resulting peak size in area units for all analytically significant peaks.

11.2.4 If the responses for the peaks exceed the working range of the system, dilute an additional 20-mL aliquot of the sample with reagent water, adjust the pH to 12 with NaOH, and reanalyze according to Sect. 11.1.2.

## 11.3 IDENTIFICATION OF ANALYTES

11.3.1 Identify a sample component by comparison of its retention time to the retention time of a reference chromatogram.

If the retention time of an unknown compound corresponds, within limits, to the retention time of a standard compound, then identification is considered positive.

11.3.2 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.3.3 Identification requires expert judgment when sample components are not resolved chromatographically. When peaks obviously represent more that one sample component (i.e., broadened peak with shoulder(s) or vallies between two or more maxima, or any time doubt exists over the identification or a peak on a chromatogram, appropriate alternative techniques, to help confirm peak identification, should be used. For this method, the use of the PDA-UV detector affords the analyst the option of using a secondary wavelength for the analysis of the questionable identification. The response ratio for a compound of interest at two wavelengths may be determined from standards of known purity. If the wavelength response ratio and the retention time matches a given unknown to a method analyte, more certainty may be assigned to the identification of the unknown. If this method of compound confirmation is employed, each analyst will need to determine the wavelength response ratio for each analyte. Table 3 lists suggested alternative wavelengths for each analyte in the scope of the method. An alternative LC column may be used to separate and confirm the identification of unknown peaks. A suggested alternative column is described in Sect. 6.4.2.

## 12. DATA ANALYSIS AND CALCULATIONS

12.1 Calculate analyte concentrations in the sample from the response for the analyte using the calibration procedure described in Sect. 10.

12.2 Calculate the amount of sample analyte injected from the peak response using the calibration curve or calibration response factor determined in Sect. 10.2. The concentration (C) in the sample can be calculated from Equation 1.

$$C\ (\mu g/L) = \frac{(A)(V_t)}{(V_i)(V_s)} \qquad \text{Equation 1.}$$

where:

A = Amount of standard injected (ng)

$V_i$ = Volume of standard injected (mL)
$V_t$ = Volume of sample injected (mL)
$V_s$ = Volume of water sample (mL)

## 13. METHOD PERFORMANCE

13.1 In a single laboratory, analyte recoveries from reagent water were determined at two concentration levels. Results were used to determine analyte MDLs (5) and demonstrated method range. Analyte MDLs and analyte recoveries and standard deviations about the percent recoveries at one concentration are given in Table 2.

13.2 In a single laboratory, analyte recoveries from dechlorinated tap water and ground waters were determined at one concentration level, 10 ug/L. Results were used to demonstrate applicability of the method to different tap and ground water matrices. Analyte recoveries from tap water and ground water are given in Table 4. MDLs calculated from results of analyses of six 100 mL reagent water samples at 0.5 µg/L concentrations for each analyte are listed in Table 5.

## 14. POLLUTION PREVENTION

14.1 This method utilizes the new in-line liquid-solid extraction technology which requires the use of very small quantities of organic solvents. This feature eliminates the hazards involved with the use of large volumes of potentially harmful organic solvents needed for conventional liquid-liquid extractions. Also, this method uses no derivatizing reagents, which are toxic or explosive, to form gas chromatographable derivatives. These features make this method much safer for use by the analyst in the laboratory and a great deal less harmful to the environment.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical Management for Waste Reduction," available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

## 15. WASTE MANAGEMENT

15.1 Due to the nature of this method, there is little need for waste management. No large volumes of solvents or hazardous chemicals are used. The matrices of concern are finished drinking water or source water. However, the Agency requires that laboratory waste management practices be consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult "The Waste

Management Manual for Laboratory Personnel," also available from the American Chemical Society at the address in Sect. 14.2.

## 16. REFERENCES

1. Glazer, J.A., Foerst, D.L., McKee, G.D., Quave, S.A., and Budde, W.L., Environ. Sci. Technol. 15, 1981, pp. 1426-1435.

2. "Pesticide Methods Evaluation," Letter Report #33 for EPA Contract No. 68-03-2697. Available from U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

3. ASTM Annual Book of Standards, Part 11, Volume 11.02, D3694-82, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 86,1986.

4. Giam, C.S., H.S. Chan, and G.S. Nef. "Sensitive Method for Determination of Phthalate Ester Plasticizers in Open-Ocean Biota Samples," Analytical Chemistry, 47, 2225 (1975).

5. 40 CFR, Part 136, Appendix B.

## 17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

### TABLE 1. RETENTION TIMES FOR METHOD ANALYTES

| Analyte | Group | Retention Times[a] (minutes) Primary Column | Confirmation Column |
|---|---|---|---|
| Picloram | (A) | 19.0 | 12.8 |
| 5-Hydroxydicamba | (A) | 19.7 | 13.5 |
| Chloramben | (A) | 21.1 | 14.8 |
| 4-Nitrophenol | (B) | 21.6 | 5.0 |
| Dicamba | (A) | 24.0 | 18.2 |
| Bentazon | (A) | 25.2 | 19.5 |
| MCPA | (B) | 25.5 | 20.1 |
| 2,4-D | (A) | 25.6 | 20.1 |
| 3,5-Dichloro-benzoic acid | (B) | 26.7 | 21.3 |
| MCPP | (B) | 27.2 | 21.8 |
| Dichloroprop | (A) | 27.3 | 21.8 |
| 2,4,5-T | (B) | 27.5 | 22.4 |
| 2,4-DB | (B) | 28.0 | 22.8 |
| 2,4,5-TP | (A) | 29.2 | 23.9 |
| Acifluorfen | (A) | 30.7 | 25.5 |
| Dinoseb | (B) | 32.8 | 27.7 |
| Pentachlorophenol | (B) | 33.4 | 28.3 |

[a] Columns and analytical conditions are described in Sect. 6.4.1 and Sect. 6.4.2.

## TABLE 2. SINGLE LABORATORY ACCURACY, PRECISION AND METHOD DETECTION LIMITS (MDLS) FOR ANALYTES FROM REAGENT WATER [(a)]

|  | MDL | Concentration | Reagent Water |  |
|---|---|---|---|---|
| Analyte | µg/L[b] | µg/L | R[c] | $S_R$[d] |
| Acifluorfen | 1.7 | 10.0 | 104 | 1.7 |
| Bentazon | 4.6 | 10.0 | 126 | 14.6 |
| Chloramben | 3.1 | 10.0 | 83 | 10.0 |
| 2,4-D | 1.3 | 10.0 | 112 | 4.2 |
| 2,4-DB | 1.9 | 10.0 | 92 | 5.9 |
| Dicamba | 2.1 | 10.0 | 104 | 6.6 |
| 3,5-Dichlorobenzoic acid | 2.1 | 10.0 | 94 | 6.7 |
| Dichlorprop | 1.7 | 10.0 | 108 | 5.4 |
| Dinoseb | 1.5 | 10.0 | 97 | 4.8 |
| 5-Hydroxydicamba | 2.2 | 10.0 | 132 | 7.0 |
| MCPA | 0.8 | 10.0 | 93 | 2.5 |
| MCPP | 1.7 | 10.0 | 95 | 5.5 |
| 4-Nitrophenol | 1.2 | 10.0 | 95 | 4.0 |
| Pentachlorophenol (PCP) | 1.6 | 10.0 | 99 | 5.2 |
| Picloram | 0.5 | 10.0 | 104 | 1.7 |
| 2,4,5-T | 1.3 | 10.0 | 93 | 4.1 |
| 2,4,5-TP | 1.8 | 10.0 | 90 | 5.8 |

[a] Data represent the average of 6-7 samples. Sample volume = 20 mL.

[b] MDL = method detection limit; defined in Appendix B to 40 CFR Part 136 - Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11.

[c] R = average percent recovery.

[d] $S_r$ = standard deviation of the percent recovery

## TABLE 3. CONFIRMATION WAVELENGTHS AND AREA RESPONSE RATIOS FOR METHOD ANALYTES.

| Analyte | Confirmation Wavelength (nm) | Area Response Ratio[a] |
|---|---|---|
| Acifluorfen | 293 | 1.72 |
| Bentazon | 240 | 1.08 |
| Chloramben | 214 | 0.61 |
| 2,4-D | 285 | 4.02 |
| 2,4-DB | 285 | 5.93 |
| Dicamba | 220 | 0.66 |
| 3,5-Dichlorobenzoic acid | 285 | 5.15 |
| Dichlorprop | 285 | 4.07 |
| Dinoseb | 268 | 0.48 |
| 5-Hydroxydicamba | 293 | 1.89 |
| MCPA | 285 | 6.66 |
| MCPP | 285 | 6.49 |
| 4-Nitrophenol | 310 | 0.56 |
| Pentachlorophenol (PCP) | 290 | 5.65 |
| Picloram | 223 | 0.82 |
| 2,4,5-T | 290 | 4.00 |
| 2,4,5-TP | 293 | 3.84 |

[a] - Area Response Ratio = Peak Area for 230 nm / Peak Area for Conf. Wavelength

**TABLE 4. SINGLE LABORATORY PRECISION AND ACCURACY DATA FROM TAP WATER AND GROUND WATER[a]**

| | Dechlorinated Tap Water | | Ground Water | |
|---|---|---|---|---|
| Analyte | $R^b$ | $S_R^c$ | $R^b$ | $S_R^c$ |
| Acifluorfen | 65.7 | ±27. | 87.3 | ±17. |
| Bentazon | 86.1 | ± 6.0 | 90.1 | ± 9.0 |
| Chloramben | 100 | ± 5.5 | 88.2 | ± 5.9 |
| 2,4-D | 117 | ± 9.6 | 105 | ± 8.1 |
| 2,4-DB | 91.2 | ± 7.0 | 97.2 | ± 6.1 |
| Dicamba | 94.3 | ± 6.1 | 86.0 | ± 7.7 |
| 3,5-Dichlorobenzoic acid | 90.2 | ± 7.2 | 92.1 | ± 5.5 |
| Dichlorprop | 92.9 | ± 6.1 | 98.3 | ±10. |
| Dinoseb | 94.1 | ± 6.2 | 91.2 | ± 4.1 |
| 5-Hydroxydicamba | 110 | ± 5.5 | 108 | ± 7.0 |
| MCPA | 92.7 | ± 5.0 | 85.2 | ± 5.7 |
| MCPP | 91.4 | ± 7.7 | 84.3 | ± 5.9 |
| 4-Nitrophenol | 89.2 | ± 10. | 103 | ± 3.4 |
| Pentachlorophenol (PCP) | 102 | ± 4.2 | 92.6 | ± 9.1 |
| Picloram | 99.0 | ± 4.9 | 84.3 | ± 5.8 |
| 2,4,5-T | 88.2 | ± 7.8 | 90.0 | ± 6.2 |
| 2,4,5-TP | 90.3 | ± 5.9 | 77.8 | ± 8.9 |

[a] - Average of six samples fortified at 10 µg/L

[b] - Mean percent recovery, corrected for background levels

[c] - Standard deviation of the mean percent recovery

## TABLE 5. SINGLE LABORATORY RECOVERY AND PRECISION DATA AND METHOD DETECTION LIMITS (MDLS) FOR ANALYTES FROM REAGENT WATER[A]

| Analyte | MDL µg/L[b] | Concentration µg/L | Reagent Water $R^c$ | Reagent Water $S_r{}^d$ |
|---|---|---|---|---|
| Acifluorfen | 0.40 | 0.5 | 114 | 23.7 |
| Bentazon | 0.12 | 0.5 | 91 | 7.3 |
| Chloramben | N.R. | 0.5 | N.R. | N.R. |
| 2,4-D | 0.34 | 0.5 | 121 | 20.2 |
| 2,4-DB | 0.31 | 0.5 | 99 | 18.5 |
| Dicamba | 0.24 | 0.5 | 80 | 14.1 |
| 3,5-Dichlorobenzoic acid | 0.38 | 0.5 | 105 | 22.5 |
| Dichlorprop | 0.33 | 0.5 | 110 | 19.4 |
| Dinoseb | 0.26 | 0.5 | 99 | 15.5 |
| 5-Hydroxdicamba | N.R. | 0.5 | N.R. | N.R. |
| MCPA | 0.35 | 0.5 | 124 | 21.0 |
| MCPP | 0.19 | 0.5 | 125 | 11.1 |
| 4-Nitrophenol | N.R. | 0.5 | N.R. | N.R. |
| Pentachlorophenol (PCP) | 0.15 | 0.5 | 93 | 8.6 |
| Picloram | N.R. | 0.5 | N.R. | N.R. |
| 2,4,5-T | 0.21 | 0.5 | 80 | 12.7 |
| 2,4,5-TP | 0.37 | 0.5 | 77 | 21.7 |

a Data represent the average of six samples. Sample Volume = 100 mL

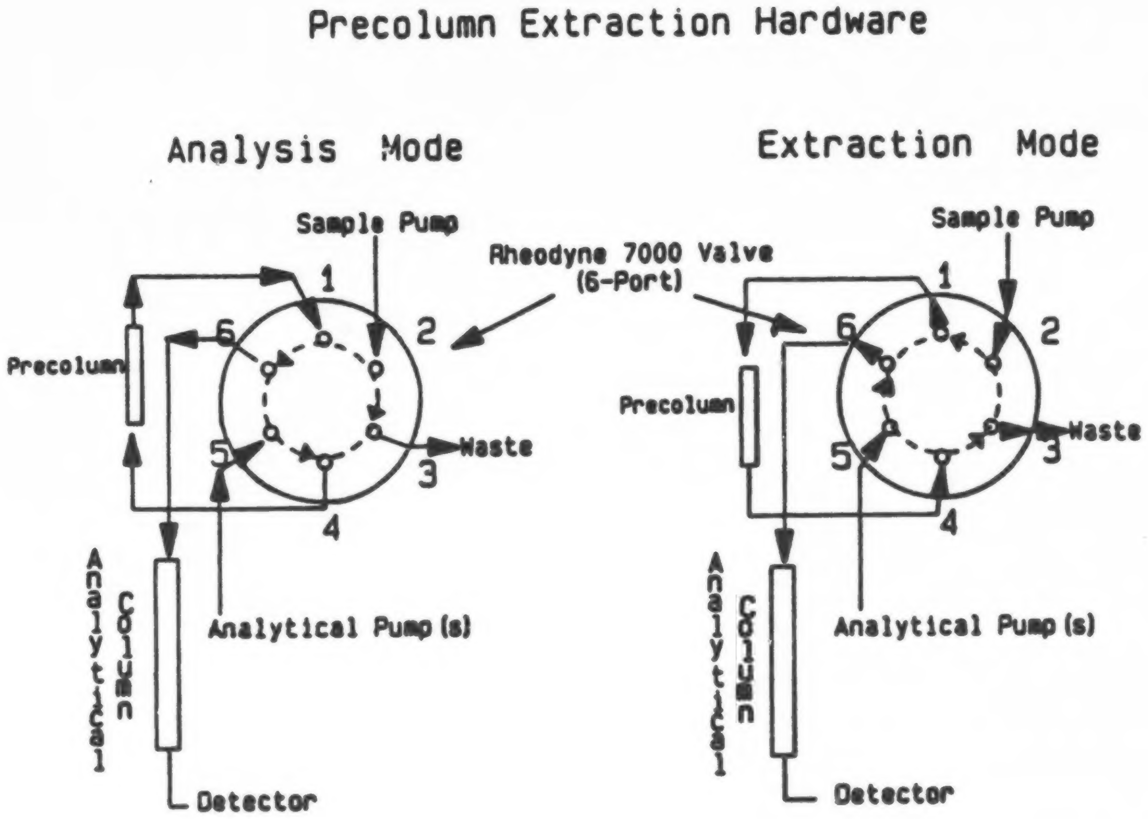
b MDL = Method detection limit; defined in Appendix B to 40 CFR 136 - Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11.

c R = Average percent recovery.

d $S_r$ = Standard deviation of the percent recovery.

N.R. = Not Recovered.

FIGURE 1. SCHEMATIC DIAGRAM OF SAMPLE CONCENTRATION AND ANALYTICAL HPLC HARDWARE


Precolumn Extraction Hardware
Analysis Mode
Extraction Mode
Sample Pump
Rheodyne 7000 Valve (6-Port)
Sample Pump
1
2
3
4
5
6
Precolumn
Waste
Analytical Column
Analytical Pump(s)
Detector
1
2
3
4
5
6
Precolumn
Waste
Analytical Column
Analytical Pump(s)
Detector

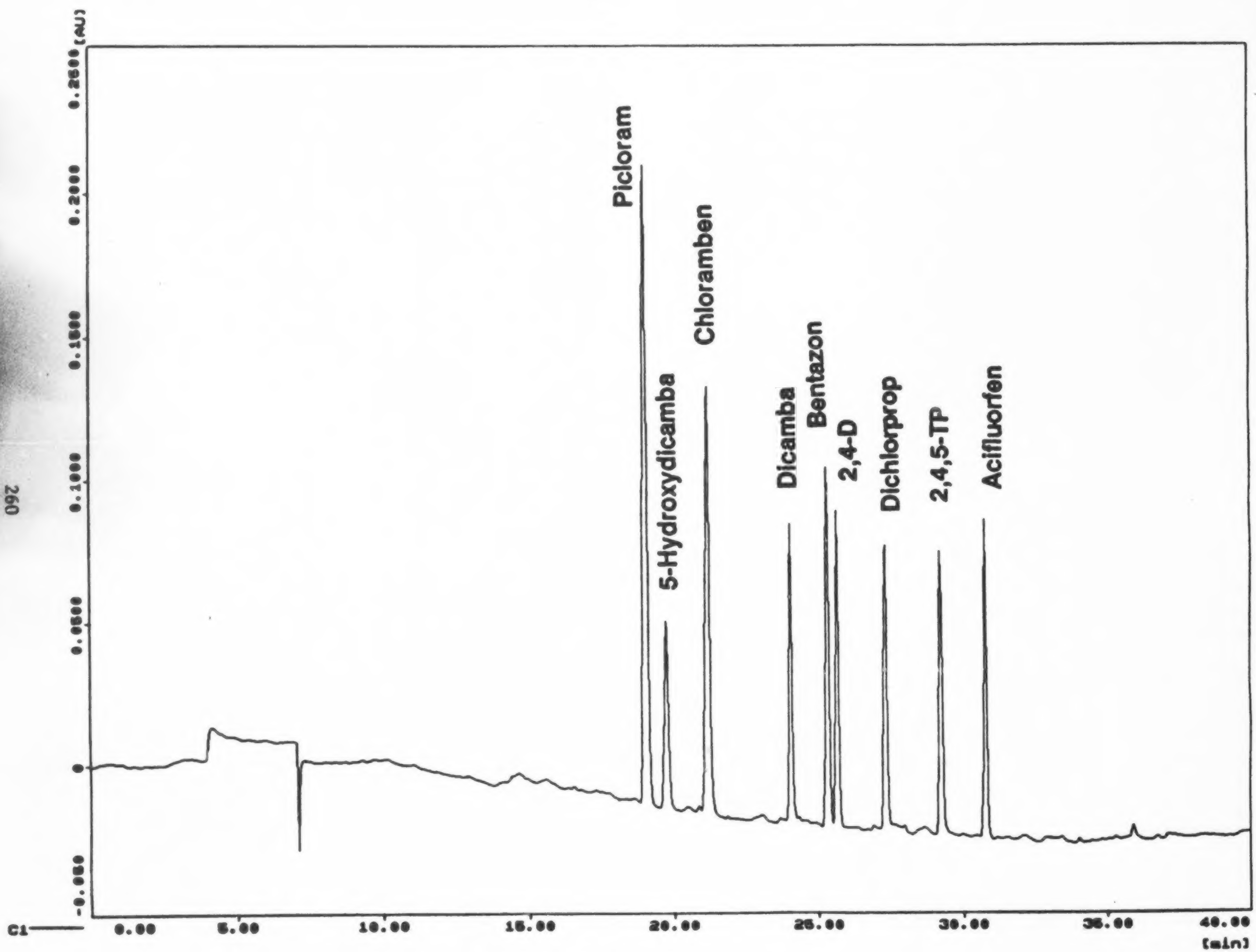


FIGURE 2. TYPICAL HPLC CHROMATOGRAM OF GROUP A ANALYTES

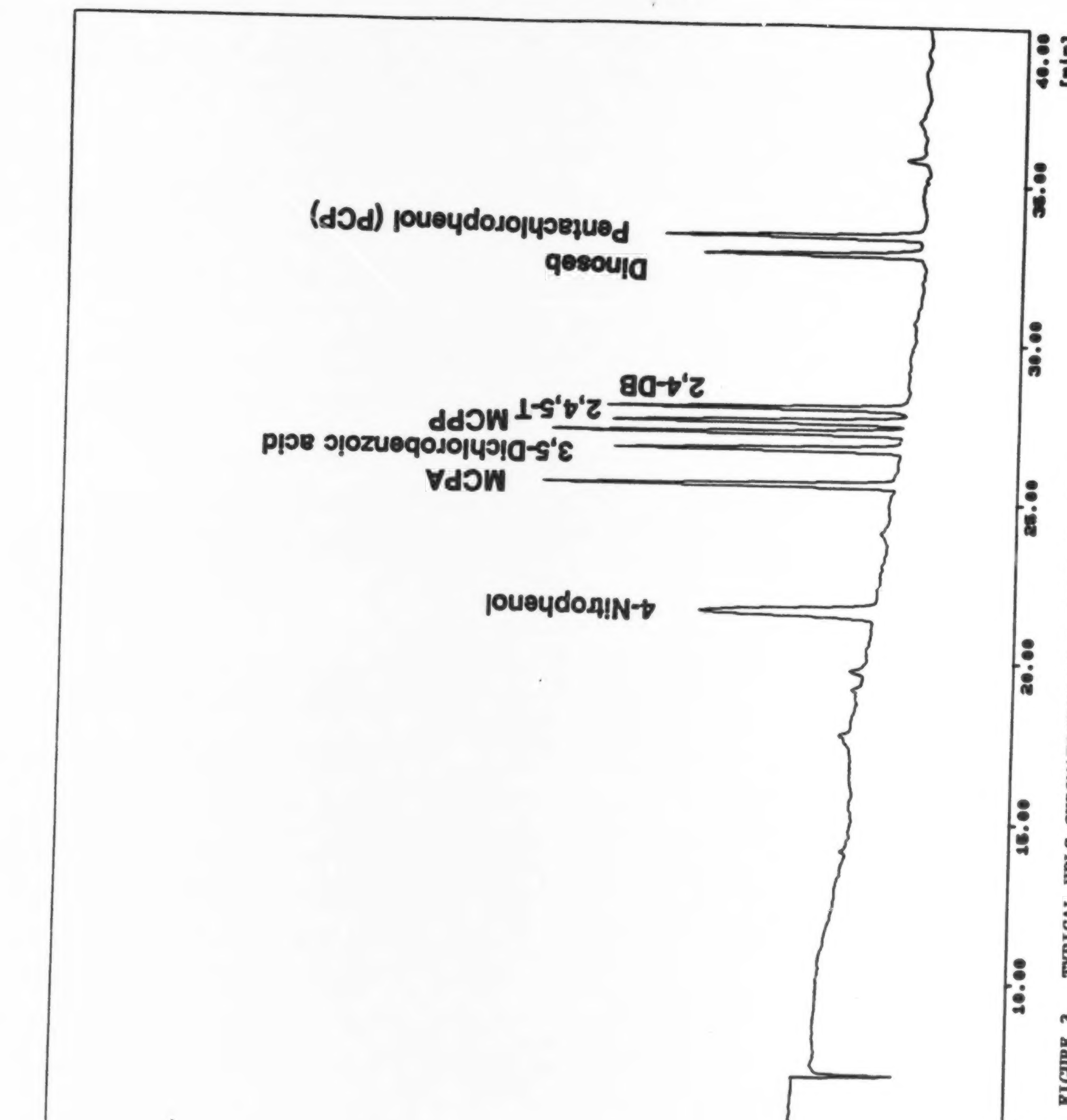


FIGURE 3. TYPICAL HPLC CHROMATOGRAM OF GROUP B ANALYTES